HUMAN CALORIMETRY

II. THE AVERAGE TEMPERATURE OF THE TISSUES OF THE BODY [1]

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THREE FIGURES

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It has been generally tacitly assumed that the average temperature of all the tissues of the human body is very close to the 37°C. or thereabouts, registered by a rectal or an oral thermometer. It is known, however, that the surface of the body is at a temperature considerably lower than this. In normal surroundings, say at 25°C., the average temperature for the whole skin surface of a lightly clothed man will be in the neighborhood of 33°C., the average being a mean of widely different temperatures at different parts of the body surface. At the feet the temperature may be as low as 27°C., while on the abdomen it may approach 35 or 36°C. The temperature, of course, rises as we advance from the skin to the interior. The experiments of Bazett and McGlone ('27) showed that in the forearm and thigh, the constant internal temperature was not reached until a depth of about 2.5 cm. was reached. Those of Benedict and Slack ('10), on the temperatures at different depths in the rectum and vagina, showed that constancy was not reached until a depth of 5 to 7 cm. In the view of these facts, the assumption that the average temperature is close to 37°C. cannot be made without investigation.

In direct calorimetry the knowledge of the true average temperature of the body is of great importance. A calorimeter

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measures the heat eliminated from the body, and if from this the heat production in the same period is to be inferred, the heat stored or lost by the body in changing its average temperature must be known. How large this item in the heat balance may be, and how great is the uncertainty in its calculation has been fully recognized by workers in calorimetry. It is discussed by Benedict and Carpenter ('10). It may be pointed out that in the case of a 70 kg., man, a rise of 0.2°C. in body temperature, not uncommonly occurring in the space of an hour, corresponds to a storage of nearly 12 kilocalories of heat in the body. This would amount to about 15 per cent of the normal basal heat production of the man in that period, and there might be a 15 per cent difference between direct and indirect heats. It was recognized by these authors that a research was very necessary on the topographical distribution of temperature in the body and its fluctuations, and that until it was made, it had to be assumed that fluctuations in body temperature as measured by a rectal thermometer are a true measure of those of the true average temperature of the whole body.

Benedict and Slack ('11) made simultaneous measurements of the temperatures in the rectum, the vagina, and in natural and artificial cavities, such as the axilla, the groin, and between the closed hands. They found a fairly close parallelism between the fluctuations of the temperatures of these points and concluded that "aside from the skin temperature, a rise or fall in rectal temperature is accompanied by a corresponding rise or fall in temperature of all other parts of the body." The use of the rectal temperature alone therefore appeared justified.

There is strong evidence, however, that grave errors exist in making the calculation of heat storage or loss from the rectal temperature alone. Thus it is claimed that the respiration calorimeter at the Russell Sage Institute of Pathology measures oxygen consumption and heat with an average error of 1.6 per cent and 0.9 per cent, respectively (Riche and Soderstrom, '15). Yet with normal subjects, all that could be said

was that "of a total of 28 periods in 17 the difference between direct and indirect heats was less than 5 per cent and only 6 showed more than 10 per cent difference." In the case of our own calorimeter in Rochester, the difference between direct and indirect heats in alcohol checks is on the average 2.9 per cent, while with human subjects, using rectal temperature alone for calculation, the average discrepancy is about 8 per cent, and cases occur where it is as much as 20 per cent. There is also the fact that changes in rectal temperature occur often which are so rapid that it is not conceivable that corresponding changes could have occurred in the whole body, the extra heat generation necessary for such a total body temperature rise being too great. Also it is well known that in typhoid and malarial fevers the rectal temperature may fall, while the surface temperature rises and vice versa. In the same series of papers, Gephart and DuBois ('15) made an attempt to use the surface temperatures measured at three or four points on the surface to improve the agreement, but with little success.

With the respiration calorimeter now in operation at Rochester, New York (Murlin and Burton, '35), data on a series of 1 hour calorimetry periods are available, in which in addition to the rectal temperature average temperatures of large areas of the surface of the body have been recorded by the method of resistance thermometry described by Burton ('34 a). These make possible a statistical examination of the possibility of using the surface temperatures as well as rectal temperature in calculating the average temperature of the body.

Theoretical considerations. The temperatures that Benedict and Slack excluded from their generalization, namely, the skin temperature, are in all probability of much greater significance than those they selected in estimating the average temperature of the body. The location of their thermometers was such that, naturally or artifically, the outflow of heat from these points was greatly reduced. In these circumstances, the temperature must approach that of the interior of the body,

as shown by the fact that all of their recorded temperatures lay above 36°C. What their results demonstrate is that the rectal temperature gives a reliable estimate of the temperature of the deep tissues of the body.

The magnitude of that portion of the body that lies within 2.5 cm. of the surface, the distance to which the gradient certainly extends, is surprisingly high, as a simple geometrical calculation shows. Suppose that the limbs, as the lower legs, are treated as truncated cones of mean diameter D cm. and of uniform density. The percentage of the volume that lies within 2.5 cm. of the surface is then given by the formula—

$$100\left(1-\frac{(D-5)^2}{D^2}\right)$$

In table 1 the calculation has been made from measurements on the author. The second column gives the mean diameter,

TABLE 1

Calculation of proportion of body affected by surface temperature

PART	MEAN DIAMETRE, CM.	PER CENT PART NEAR SURFACE	PART AS PER CENT OF WHOLE	PER CENT OF WHOLE NEAR SURFACE
Feet	....	80.0	3.6	2.9
Lower legs	9.3	78.5	10.5	8.3
Upper legs	14.4	57.6	23.2	13.3
Trunk	26.3	34.5	42.7	14.7
Upper arms	8.9	81.0	6.7	5.4
Lower arms	7.3	90.0	4.6	4.1
Hands	....	80.0	1.7	1.4
Head	18.0	62.0	7.0	4.3
		Total percentage of body near surface		54.4

the third column the percentage of the part of the body that is within 2.5 cm. of the surface. The head is treated as a sphere, for which the formula is—

$$100\left(1-\frac{(D-5)^3}{D^3}\right)$$

The next column gives, from the figures of Braune and Fischer (1889), the weight, or volume, of the limb as a percentage of the whole body. The last column is the result of multiplying

together the third and fourth columns and gives the percentage which that peripheral tissue forms of the whole body. The total of the last column states the surprising fact that 54.4 per cent of the body of the author is within 2.5 cm. of the surface. The calculation has little significance except to emphasize the fact that a very large proportion of the whole body is at a temperature considerably lower than the internal temperature. For a small subject a similar calculation gave 58 per cent, for obese subjects it might be less than 50 per cent. Obviously, the temperature of the surface must be given considerable weight in calculating the average temperature of the whole body.

The manner in which the internal and the surface temperatures are to be weighted in finding the average temperature of these peripheral tissues depends upon the nature of the thermal gradients existing in them. The work of both Bazett and McGlone ('27) and of Benedict and Slack ('11) shows that the distribution of temperature is not linear, but in general parabolic, the gradient being steeper near the surface, a form of distribution that agrees with theoretical predictions for a cylinder in which heat is being uniformly generated (Burton, '34 b). Simple cases may be taken assuming different temperature distributions, to illustrate how the average temperature T_A depends on the internal temperature T_i and surface temperature T_s. a) For a plane slab with linear distribution of temperature, of course—

$$T_A = .5\,T_i + .5\,T_s$$

b) For a cylinder with linear distribution of temperature (a case not physically possible)—

$$T_A = .33\,T_i + .67\,T_s$$

c) For a cylinder in which the distribution of temperature is parabolic throughout—

$$T_A = .5\,T_i + .5\,T_s$$

Once more the average is the arithmetic mean of the internal and external temperature. This is evidently the best

way to calculate the average temperature of the limbs, being theoretically sound as well as the simplest possible. For the trunk, however, this method of averaging might be thought to give undue weight to the surface temperature, since here there is a large proportion (65 per cent or so) that lies more than an inch from the surface. We might assume that the gradient extends to a depth of 1 inch and that inside there is uniform temperature. This distribution could arise only if in the inner region there were very effective transfer of heat, or if within it there was a relatively small amount of heat generated. The first of these conditions is probably true, the second is hardly correct, since the visceral organs are large generators of heat. It is more likely that a parabolic distribution holds to greater depths than 1 inch. The temperature of the stomach is known to be slightly higher (0.1°C.) than that of the rectum (Rancken and Tigerstedt, '08).

A calculation has been made to find the formula for the average temperature, assuming that the distribution is parabolic up to a depth of 2 inches from the surface, and uniform at the temperature T_i inside. The result is—

$$T_A = .8\,T_i + .2\,T_s;$$

using 1 inch as the critical depth, it would be .89 T_i plus .11 T_s; using 3 inch, .71 T_i plus .29 T_s. The figures .8 T_i plus .2 T_s are used for the trunk in the next calculation, which consists in taking the formula for the average temperature of each part of the body, assuming in each case that the internal temperature is the rectal temperature T_R, and multiplying by the fraction that the part forms of the weight of the whole body to get the contribution of that part to the final formula. Addition of the contributions for all the parts gives a final formula involving all the temperatures, T_1, T_2, T_3 etc., measured, of the form:

$$T_A = A_1\,T_1 + A_2\,T_2 + A_3\,T_3 + \ldots.$$

Table 2 gives, in the second column, the calculated values of the coefficients A_1, A_2, appropriate to the temperatures measured at the various places listed in the first column. If

it is not possible or desirable to measure temperatures at all the places mentioned, the formula may be simplified for use with a smaller number of temperature measurements. For instance, the surface temperature of the upper arm may be assumed to be the mean of those of the lower arm and of the trunk. Its coefficient (0.033) is then divided equally between the coefficients for the trunk and for the lower arm, as shown by the arrows in the table. (Mathematically, if, in the formula T_2 is put equal to $\frac{T_1+T_3}{2}$, there results:

$$T_A = (A_1 + \tfrac{1}{2} A_2)\, T_1 + (A_3 + \tfrac{1}{2} A_2)\, T_3 + \ldots .)$$

TABLE 2

POSITION WHERE TEMPERATURE IS MEASURED	COEFFICIENT IN FORMULA	REPARTITION OF COEFFICIENTS	COEFFICIENT IN SIMPLIED FORMULA	TEMPERATURES USED IN SIMPLIFIED FORMULA
Rectal temperature	.59	→	.64	Rectal
Oral temperature	.05	→		
Surface of lower leg	.07	→	.13	Surface of lower leg
Surface of upper leg	.12	→		
Surface of trunk	.09	→	.18	Surface of trunk
Surface of head	.02	→		
Surface of upper arm	.03	→		
Surface of lower arm	.03	→	.05	Surface of lower arm

The simplified formula in the third column applies when the surface temperatures on the trunk, on the lower leg, and on the lower arm are used. It may be recast into the form—

$$T_A = .64\, T_s + .36\, T_s$$

where T_s is an average surface temperature given by—

$$T_s = .50\, T_1 + .36\, T_3 + .14\, T_3$$

where T_1 equals average temperature on surface of trunk; T_2 equals average temperature on lower leg; T_3 equals average temperature on lower arm. It is seen that the rectal temperature carries 64 per cent of the average.

This calculation may appear to be based upon so many assumptions that it can command little confidence, but it is found that quite considerable changes may be made in those assumptions without changing greatly the resulting formula. It is remarkable that this theoretical formula, which was calculated before any experimental verification had been made, so nearly approximates to the one found purely by experiment. It suggests that the human body may be more a fit subject for mathematical equations than has been realized.

Experimental determination. The material for the experimental determination of the formula for 'mixing' the rectal and surface temperatures was provided by a series of experiments in the respiration calorimeter upon nine subjects, seven of them male, two female, providing the necessary data of forty 1-hour periods. In nineteen of these periods the subjects were in the basal state, the rest of the periods followed 2 and 3 hours after the ingestion of a carbohydrate or a meat meal. The details of the experiments and the bearing of the results on the effect of ingestion of food are discussed in another paper (Burton and Murlin, '35). The data are thus of a character taken under conditions so diverse that the resulting conclusion as to the average temperature of the body has an 'omnibus' value, and is applicable to calorimetry in general. A result of general application was desired rather than one, perhaps of more accuracy, but applying only to a given subject under a given set of conditions.

Given a sufficient number of determinations by the respiration calorimeter of the heat stored or lost by the body in hour periods, and of the changes in rectal temperature and in surface temperature occurring in the same periods, it is obviously possible to determine experimentally the best coefficient for the rectal and surface temperatures. Assume that the formula is of the type—

$$T_A = aT_R + bT_S$$

The consideration that if the rectal temperature and the surface temperature each rise by, say 1°C., the average temperature must also rise by 1°C., leads to the conclusion a plus b

equals 1; or b equals (1 — a). The equation then can be written:

$$T_A = aT_R + (1-a)\,T_S.$$

If in any period the changes in the rectal, surface and average temperatures are—

$$\Delta T_R, \Delta T_S \text{ and } \Delta T_A$$

then—

$$\Delta T_A = a\,\Delta T_R + (1-a)\,\Delta T_S$$

The change in the average temperature, ΔT_A, is found by dividing the quantity of heat stored or lost by the body in the period, equal to the difference between indirect and direct heats, by the thermal capacity of the body. The latter is assumed to be the product of the weight and an average specific heat, taken as 0.83 by general custom, i.e.,

$$\Delta T_A = \frac{\text{Indirect heat—Direct heat}}{\text{Weight} \times 0.83}$$

The problem is then to find the value of the coefficient 'a' which statistically makes the equation most nearly true for the greatest number of cases. The value ΔT_S is calculated from the three surface temperatures measured, namely, that over the trunk, over the lower leg, and over the forearm, by the formula: T_S equals .50 T_1 plus .36 T_2 plus .14 T_3, which weights the respective temperatures according to the mass of tissue beneath them. (Weighting the temperatures according to area would give relatively more weight to the leg and forearm.)

From the results of any given period, the value of 'a' best fitted to that set of values can be found from the relation—

$$a = \frac{\Delta T_A - \Delta T_S}{\Delta T_R - \Delta T_S}$$

It might be thought that this ratio should be calculated for each period and the resulting values of 'a' averaged. This, however, would give undue weight to those determinations that are least able to give a reliable value of the coefficient. For suppose that in a period, the change in rectal and in surface temperatures, though both considerable, are equal.

Then, no matter how the two are weighted in the average (i.e., no matter what the value of 'a' chosen), the resulting change in average temperature is the same quantity. Such an experiment can give no information as to the value of the 'mixing coefficient' 'a.' Mathematically, the same thing is stated when we observe that if the denominator $(T_R - T_S)$ is a small quantity, very slight changes in its value, perhaps due to the errors of measurement of the temperatures, produce very large differences in the value of 'a.' The theory of least squares indicates that the best value of 'a' is obtained by the formula—

$$a = \frac{\Sigma XY}{\Sigma Y^2}$$

where X equals $(T_A - T_S)$ and Y equals $(T_R - T_S)$. In the sums here, those experiments in which the quantity Y is small are automatically given the least weight.

Since the quantity ΔT_A obtained from the difference between indirect and direct calorimetry, is subject to the errors of the respiration calorimeter, the problem is essentially soluble only statistically. It was therefore imperative, as in all statistical work, that all results obtained should be included unless there were reasons for rejection other than that the values of the coefficient 'a' appeared to diverge from the average. Such valid reasons were considered to be wildly improbable values of the R.Q., indicating that the oxygen consumption might be incorrectly measured, or obviously incorrect temperature measurements which fluctuated abnormally. In the whole series, only one set of three periods on one subject has been rejected for such reasons. In table 3 the results of the forty periods are tabulated. The last three columns record the progress of the sums ΣY^2 and ΣXY and of the statistically most suitably value of the coefficient 'a.' Several cases will be seen in the table where no possible positive value of the coefficient would make the observed change of average temperature derivable from the change in rectal and in surface temperatures. Yet when the method of taking the average is used, these periods prove to have little effect upon the final value for the coefficient.

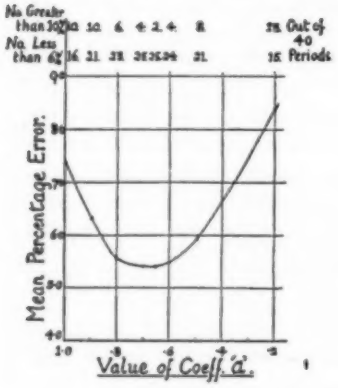
TABLE 3

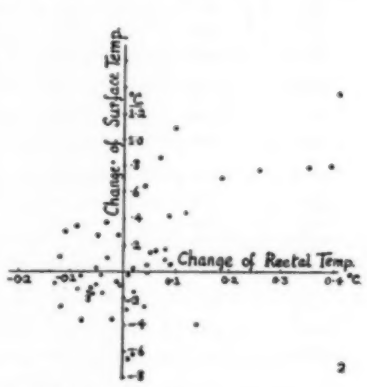
DATE 1934	THERMAL CAPACITY OF SUBJECT. CALS./°C.	ΔH = INDIRECT HEAT—DIRECT HEAT	ΔT_A	ΔT_B	ΔT_S	Y	X	ΣY^2	ΣXY	s
4–16	71.9	— 2.79	— .039	—. 11	+ .30	— .41	— .34	.168	.139	.83
	71.9	— 1.09	— .015	— .03	+ .097	— .127	— .112	.184	.153	.83
5–2	46.5	— 1.36	— .030	+ .08	+ .081	.00	+ .051	.184	.153	.83
	46.5	— 3.87	— .085	— .01	— .086	+ .076	+ .001	.189	.153	.81
	46.5	+ 2.94	+ .063	+ .09	+ .411	— .321	— .348	.292	.265	.91
	46.5	— 2.10	— .045	— .005	— .292	+ .287	+ .247	.375	.336	.90
5–7	46.5	— 3.78	— .081	+ .14	— .413	+ .553	+ .332	.678	.519	.77
	46.5	— 4.84	— .104	— .01	+ .268	— .272	— .372	.750	.622	.83
	46.5	+ 13.00	+ .280	+ .09	+ .045	+ .045	+ .235	.752	.638	.85
	46.5	— 3.04	— .065	— .13	— .093	— .037	+ .028	.753	.637	.85
5–11	51.5	— 6.79	— .132	— .06	— .172	+ .112	+ .040	.766	.641	.84
	51.5	— 5.45	— .106	— .04	— .180	+ .140	+ .074	.786	.651	.83
	51.5	+ 3.58	+ .070	— .03	+ .380	— .410	— .310	.954	.778	.82
	51.5	+ 0.52	+ .010	+ .06	+ .149	— .089	— .139	.962	.790	.82
5–16	64.8	— 10.99	— .170	— .08	— .31	+ .23	+ .14	1.015	.822	.81
	64.8	+ 11.96	+ .184	+ .04	+ .65	— .61	— .466	1.387	1.106	.80
	64.8	+ 6.43	+ .099	— .04	— .03	— .01	+ .13	1.387	1.105	.80
5–23	51.2	— 10.65	— .242	— .08	— .370	+ .290	+ .128	1.471	1.142	.78
	51.2	— 5.73	— .112	— .115	— .257	+ .142	+ .145	1.491	1.162	.78
	51.2	— 9.50	— .185	— .06	— .130	+ .070	— .055	1.496	1.158	.78
	51.2	+ 0.76	+ .015	— .05	+ .025	— .075	— .040	1.502	1.161	.77
	51.2	— 7.12	— .139	+ .02	— .164	+ .144	+ .025	1.523	1.165	.77
6–12	60.2	— 6.93	— .115	+ .04	— .272	+ .232	+ .157	1.577	1.201	.76
	60.2	+ 3.45	+ .057	+ .01	+ .004	+ .006	+ .053	1.577	1.201	.76
	60.2	— 3.83	— .064	+ .02	+ .020	+ .000	— .084	1.577	1.201	.76
5–18	45.2	— 12.15	— .269	— .06	— .221	+ .161	— .048	1.603	1.193	.75
	45.2	— 13.00	— .288	+ .02	— .623	+ .643	+ .335	2.015	1.408	.70
	45.2	— 0.58	— .013	— .05	+ .267	— .317	— .280	2.115	1.497	.71
	45.2	+ 2.98	+ .066	+ .02	— .245	+ .265	+ .311	2.185	1.579	.72
6–15	65.3	— 5.41	— .083	— .01	— .083	+ .073	.000	2.190	1.579	.72
	65.3	+ 3.74	+ .057	+ .08	+ .163	— .083	— .106	2.197	1.588	.72
	65.3	1.76	— .027	+ .055	+ .139	— .084	— .166	2.204	1.601	.73
6–21	63.0	— 14.90	— .237	— .065	— .159	+ .094	— .078	2.212	1.593	.72
	63.0	— 7.09	— .113	— .005	— .105	+ .100	— .108	2.222	1.582	.71
	63.0	+ 0.90	+ .014	+ .045	— .009	+ .054	+ .023	2.225	1.583	.72
	63.0	— 1.79	— .028	+ .075	+ .004	+ .071	— .032	2.230	1.581	.71
6–28	55.8	— 5.41	— .097	— .085	— .138	+ .053	+ .041	2.233	1.583	.71
	55.8	— 5.32	— .096	+ .045	— .046	+ .091	— .050	2.241	1.575	.70
	55.8	+ 2.17	+ .039	+ .090	— .065	+ .155	+ .104	2.265	1.591	.70
	55.8	— 6.30	— .118	+ .005	— .196	+ .201	+ .078	2.306	1.607	.70

It remains to make use of the formula thus derived, namely—

$$\Delta T_A = .70 \Delta T_R + .30 \Delta T_s$$

to calculate the change of average temperature from the change in rectal and in surface temperatures, and, using this value to calculate the heat storage or loss by the body, to see how much deviation there is then between direct and indirect heats. Using the rectal temperature alone the average discrepancy in the forty periods was 7.48 per cent, using the surface temperatures also, with 0.70 as the coefficient 'a,' this is reduced to an average discrepancy of 5.47 per cent. The





Figures 1 and 2

number of periods in which the discrepancy was more than 10 per cent is reduced from 10 to 4 and the number where it is less than 6 per cent increased from 16 to 25. *There is no doubt that by the use of the surface temperatures a great gain in the accuracy of calculating the change in average temperature has been made.*

In order to see how critical was the choice of the coefficient, the calculation of the average discrepancy has been made using other values for the coefficient, ranging from the value 1.0 (meaning that rectal temperature alone is used) to 0.2. The result is shown graphically in figure 1. Any value of the

coefficient between 0.6 and 0.75 gives the improved accuracy. It seems likely that for any one individual the curve would have a steeper fall to a minimum, the choice of the coefficient being more critical. The best value would not be the same, however, for different individuals, for the thin and the obese subject, for instance. The result is that for a large group of subjects the best value is not fixed within very narrow limits. It appears that the value of 0.65 for the coefficient is the best for this group, the gain being even more marked in the reduction of the number of large errors than in the average error. The value deduced theoretically was 0.64.

The curve also shows that the rectal temperature is the best single temperature for the calculation, the use of the surface temperatures alone leading to a very large error.

Correlation between rectal and surface temperatures. In fifteen cases out of the forty in table 3 the changes in rectal and in surface temperatures occur in opposite directions. This suggests that the correlation between the two is not very great, a conclusion which is borne out by the graph, figure 2, which includes the results of fifty-three hour periods. For small changes of temperature there is quite a large probability that the changes may occur in opposite directions, giving points in the diagram lying in the 'N.W.' and 'S.E.' quadrants. For larger changes of temperature there appears to be greater correlation, roughly to the relation—

$$\Delta T_S = 4. \Delta T_R$$

The total of all the changes in rectal temperature (without regard to sign) is 4.245°C., while the total of the changes in surface temperature that simultaneously occurred is 16.035°C. The changes in surface temperature were then on the average 3.8 times as great as the changes of rectal temperature. In the equation for the average temperature—

$$\Delta T_A = .65 \Delta T_R = .35 \Delta T_S$$

if ΔT_S is 3.8 times as great as ΔT_R, the term in the average arising from the surface temperatures is (since $\frac{.35 \times 3.8}{.65 \times 1.0}$ equals 2.0), more important than the term from the rectal tempera-

ture. Hence, *although the rectal temperature has the larger coefficient in the average, the measurement of the surface temperature is of more importance.*

This degree of correlation also suggests that if one temperature alone is to be used, either the rectal or the surface temperatures, better agreement might be obtained by substituting in the equation from the correlation equation: ΔT_S equals $3.8\ \Delta T_R$. For instance, using the rectal temperature alone, the equation becomes—

$$\Delta T_A = .65\ \Delta T_R + .35 \times 3.8\ \Delta T_R = 1.9\ \Delta T_R.$$

instead of the usual employed relation, ΔT_A equals ΔT_R. For the whole group, this gives no better average error than using the rectal temperature itself, due to the lack of correlation when the changes are small. The device would obviously be of use only if the rectal temperature changes by more than 0.2°C., no case of which is available for which the direct and indirect heat is simultaneously known. If the surface temperature alone were used the formula would be—

$$\Delta T_A = \frac{.65}{3.8}\Delta T_S + .35\ \Delta T_S = .61\ \Delta T_S.$$

The errors have been calculated using $.6 \times \Delta T_S$ alone. The resultant average error is 6.72 per cent, with eleven periods having the error greater than 10 per cent and twenty-two with errors less than 6 per cent. This is as good as, if not better than, the result of using the rectal temperature alone. This might conceivably not be true for a larger group of periods, but it is evident that the surface temperature changes may be made to give as good an indication of the changes in average body temperature as the rectal temperature changes.

The average temperature of the body tissues. Having found the value of the coefficient 'a,' the same figure can be used to find the absolute value of the true average temperature of the whole body. Taking a case of a subject in basal condition, with the following temperatures:

Rectal temperature,		$T_R = 36.95$°C.
Average surface temperature	on trunk,	$T_1 = 35.05$°C.
	on lower leg,	$T_2 = 29.60$°C.
	on lower arm,	$T_3 = 28.40$°C.

we find the average surface temperature T_s equals 31.66°C. and the average temperature for the whole body, T_A, equals 35.10°C. In this case the average for the whole body is 1.85°C. below the rectal temperature. This must obviously be taken into consideration when the relation between body temperature and metabolism is being studied.

It has been pointed out (Nasset, '32) that in the curve given by DuBois ('21) for the increase of the metabolism of febrile patients with rise of rectal temperature between 37° and 40°C., the quantity Q_{10}, calculated by the well-known formula—

$$\log Q_{10} = \frac{\log K_1 - \log K_2}{t_1 - t_2} \times 10.$$

is greater for the smaller rises of temperature, say 37° to 38°, than for the larger range, say 37° to 40°. It has been shown by the author (Burton, '34 b) and by many others that in fever the surface temperatures rise a great deal more than the rectal temperature, and, indeed, reach values very close to that temperature. This is the consequence of the increased general and peripheral circulation. At the higher rectal temperatures, then, the average temperature for the whole body, which was from 1 to 2° below the rectal temperature in the basal, afebrile, condition, is now more closely equal to the rectal temperature. The true rise of average temperature is greater than the rise of rectal temperature, so that the Q_{10} calculated from rectal temperature alone is greater than the true Q_{10}. It is obvious that the error will be of greater magnitude for the smaller changes of rectal temperature, since the surface temperature soon approximates to and never exceeds the rectal temperature. It may well be that measurements of surface temperature in fever and the use of the formula deduced for the average temperature would show that the metabolism followed the Arrhenius equation more closely than has appeared.

Measurement of the rectal temperature alone is still less adequate in experiments where the body temperature is raised by high frequency currents, by hot water or hot air baths, for here the surface temperature may actually rise above the

rectal temperature. A series of experiments was made in this laboratory in which it was attempted to raise the body temperature of normal subjects in basal condition by high frequency currents, applied by electrodes to the sole of each foot. Skin temperatures were simultaneously taken. It was not found possible to raise the rectal temperature by more than 0.7 or 0.8°C. before copious sweating and discomfort

TABLE 4

Diathermy experiment February 27, 1934

NO.	RECTAL TEMPERATURE, °C.	SURFACE TEMPERATURE °C.				AVERAGE BODY TEMPERATURE, °C.	METABOLISM, CALS./HR.
		Trunk	Lower leg	Lower arm	Average		
1	36.75	34.86	32.68	32.80	33.79	35.71	63.03
2	37.32	37.32	33.63	36.92	35.52	36.69	67.48
3	37.49	33.58	37.48	33.12	34.92	36.59	67.42
4	37.20	32.63	33.63	32.18	32.93	35.71	59.65

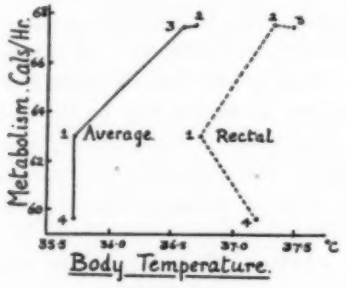


Figure 3

of the subject intervened. Before this occurred, however, the temperature of the surface of the body rose by vastly greater amounts, that of the leg becoming in some cases greater than rectal temperature and that of the trunk and arms approaching it. The metabolism was taken in ten minute periods by the Tissot-Haldane method. An example is given in table 4, and shown graphically in figure 3. In periods 1 and 2 the body temperature was rising, in 3 and 4, the current had been turned off and sweating had cooled the surface greatly, though

the rectal temperature had fallen only slightly. It will be seen that the metabolism shows little correlation with the rectal temperature, but much more with the calculated average temperature. How much the Q_{10} may be modified is shown by the fact that for points 1 and 2, using rectal temperature alone, Q_{10} is 3.3; using the average temperature, its value is 2.0. It is probable that many of the contradictory results in this field are due to the inadequacy of the rectal temperature to represent the true average temperature of the active tissues.

The specific heat of the human body. In the calculations, the value 0.83 for the average specific heat of the body has been assumed. This figure is the result of averaging the specific heats that have been found, in vitro, for various tissues, weights being given in the average according to the amount of such tissues in the body. The specific heats of tissue vary from 0.70 for fat to nearly unity for blood. A list is given with references, by Pembrey (1898). The specific heat has never been determined experimentally for a whole living man. It must obviously differ in different individuals according to the amount of subcutaneous fat. It is evident that no determination can be made until the calculation of the true average temperature can be made with certainty, and for this we have assumed the value of the specific heat. Making no assumption, but leaving it as 's,' we have—

$$\Delta T_A = \frac{\Delta H}{W.s}$$

and

$$\frac{\Delta H}{W} \times \frac{1}{s} = a\,\Delta T_R + (1-a)\,\Delta T_S.$$

Given a large series of data of simultaneous values of the temperature changes ΔT_R, ΔT_S and $\frac{\Delta H}{W}$, it is possible by the methods of statistics to find the best values not only of the coefficient 'a,' but of the specific heat 's' also. The problem is geometrically that of drawing the best plane through a set of points in three dimensions, as the problems ordinarily encountered are of drawing the best line through a set of points in two dimensions. The computation is laborious and the forty

periods here available are not sufficient to make it worth while. An alternative method is the well known one of 'successive approximation.' Having assumed the value of one of the unknowns, as we did that of the specific heat 's,' it is used to find the best value of the other unknown, in this case of the coefficient 'a.' The process is then reversed; using the value of 'a' then determined, the best value of 's' is found. If this value differs greatly from the value originally assumed, the process has to be repeated until an unchanging result is reached. Using the value 0.65 for the coefficient 'a,' the calculation of the errors has been made using values of the specific heat from 0.6 to 1.0. The perhaps surprising result is that for this group of experiments, the choice of the value of the specific heat used in the calculations is not at all critical. The figures for the average errors are:

$s = 1.0$, 5.87 per cent, $s = 0.90$, 5.55 per cent,
$s = 0.83$, 5.52 per cent, $s = 0.70$, 5.56 per cent and
$s = 0.60$, 5.59 per cent.

The figure 0.83 gives only slightly better results than the other values within quite wide limits. From the data it is not possible to determine the average value of the specific heat, except that it lies within the wide limits of 0.7 to 0.9. Rough though this determination is, it is yet the first experimental determination of the specific heat of the living human body. It is evident that, without the measurement and use of surface temperatures, no such determination even of this degree of accuracy, would be possible.

SUMMARY

The temperature of the surface of the human body is 4° or 5° lower than the interior temperature, which is not reached until a depth of several centimeters. A calculation is made showing that about 50 per cent of the body is within an inch from the surface. Account must be taken of this cooler peripheral tissue in calculating the average temperature of the body, especially for the agreement of direct and indirect calorimetry. A theoretical estimate is made of how the rectal

and surface temperatures should be combined to give the true average.

From the results of forty hour periods with the respiration calorimeter upon a group of subjects in basal and absorptive condition, the formula giving the least average discrepancy between direct and indirect heats is found experimentally. It agrees closely with that deduced theoretically, and is: average temperature equals 0.65 × rectal temperature plus 0.35 × average surface temperature. The latter temperature is found from measurements over the trunk, the lower leg and the forearm. The average error is reduced from 7½ per cent using rectal temperature alone to 5½ per cent using the formula.

The changes occurring in surface temperature are on the average three or four times as great as those of the rectal temperature, and are poorly correlated with the latter when the changes are small. The measurement of surface temperatures is equally if not more important than the rectal in the calculation of the average. By using 0.6 times the change in surface temperature alone, the results in this group are slightly better than using rectal temperature alone.

The average temperature from the body is from 1 to 2°C. lower than the rectal temperature in the basal state. In fever the two may be practically equal. Some discrepancies in the calculation of the Q_{10} for metabolism with temperature are explained, and the formula is applied to some experiments where subjects were heated by high frequency currents.

The calculation of the average specific heat of the living human body is made possible for the first time, but the data can show only that it lies between 0.7 and 0.9.

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HUMAN CALORIMETRY

III. TEMPERATURE DISTRIBUTION, BLOOD FLOW AND HEAT STORAGE IN THE BODY IN BASAL CONDITION AND AFTER INGESTION OF FOOD [1]

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FOUR FIGURES

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Interest in the subject of this research was stimulated by the preliminary report in 1933 by Essex and co-workers ('33) of studies on the blood flow to the liver and to the limbs of dogs after ingestion of food. A paper describing later work of the same authors has recently been published (Herrick et al., '34). It had generally been supposed that during digestion of food, blood was diverted from the somatic tissues to supply the viscera. As these authors state, this belief "has been so general as to give it the force of demonstrated fact." Contrary to expectation, their experiments show that there is a prolonged general increase in blood flow, measured in femoral, carotid, and mesenteric arteries and jugular vein, after ingestion of solid or liquid food.

It seemed of importance that this phenomenon should be studied in man. The method used on dogs, that of the 'thermostromuhr' of Rein ('28) with local anaesthetics, is obviously inapplicable to human subjects. Information as to blood flow must be obtained from observations of the changes in skin temperature. By the use of the 'thermal circulation index' introduced by one of us (Burton, '34 b), measurements of skin temperature may be made to yield reliable information as to the blood flow to the limbs. A study has therefore been

[1] This work has been aided by a grant from the Rockefeller Foundation.

made of the changes occurring in the rectal and surface temperatures of human subjects in basal condition and after ingestion of food. The measurements have been made simultaneously with calorimetry periods in the respiration calorimeter (described in paper I of this series, Murlin and Burton, '35) so that at the same time the heat production, heat elimination and their difference, the heat storage or loss by the body in changing its average temperature are available. The experiments furnish information in answer to the question of what happens to the extra heat produced in the body by the specific dynamic action of food.

Experimental material. In all, ten adult subjects were used, two being female. Their weights ranged from 51.2 to 85.5 kg., and their heights from 159 to 188 cm., so that they form a diversified average group. The routine of experiment was the same in the majority of the cases, those in which it differed being described later. Some experiments also were made with the subject not in the calorimeter.

The subjects came to the laboratory in basal condition, having had no food since the previous evening. They lay in the bed of the calorimeter while the various thermometers, rectal thermometer, and skin temperature thermometers on the abdomen, on the lower leg and a forearm, were placed in position. The construction and use of these thermometers has already been described, in the first paper of this series (Murlin and Burton, '35) and in detail in an earlier paper (Burton, '34 a). Over them light clothing was worn, of an amount that had been found in previous experiments to make the subject 'comfortable' at the temperature of the chamber (about 23°C.). In the case of the male subjects, this was usually shirt and trousers, covered by a laboratory coat. The chamber having been closed, air and water were circulated in the systems for about an hour in a preliminary period in which thermal and gaseous equilibrium was established. Two calorimetry periods of an hour each followed. The subject then emerged and ate the meal, light exercise being found desirable for the relaxation of the subject making possible

the 3 further hours in the calorimeter. About 20 minutes after the meal, the subject reentered the chamber and a preliminary period was followed by two more calorimetry periods, starting an hour after the meal was finished. Two periods in the basal state, and two, the second and third hours after the meal, were thus obtained. The measurements of body temperature, taken every 5 minutes throughout, extend over longer periods, nearly 3 hours in each case. In one experiment the subject remained an extra hour, so that three periods were obtained after the meal, with temperatures followed for 4 hours.

The normal variation between individuals, as well as the errors of calorimetry, render any conclusion based on an individual experiment of little value. Accordingly, in the results that follow, the average of nine similar experiments is given the most weight in the conclusions. Lack of space forbids the listing of the details of all the experiments.

Nature of meals taken. In five experiments of the first group the meal was mainly of carbohydrate, in the other four almost exclusively of protein. The carbohydrate consisted partly of starchy foods like Cream of Wheat, cornstarch, and bread, partly of sugars, cane and Karo syrup, partly of fruits, such as peaches and orange or grape fruit juice. The carbohydrate equivalent of the amount ingested was from 180 to 320 gm. The protein meals were of lean chopped beef, in amounts from 200 to 434 gm. The study is a preliminary one of the effect of food, in general, on a number of subjects. For accurate conclusions upon the comparison of the effects of particular foods, a number of experiments upon the same individual would be necessary with more carefully controlled quantities ingested.

Heat storage. If the heat produced in the body, given by the 'indirect heat' calculated from the oxygen consumption, exceeds the heat eliminated in the same period, the 'direct heat' measured by the calorimeter, heat has been stored by the body, its average temperature rising. Thus in what follows, the quantity called 'heat storage' is defined as the in-

direct heat minus the direct heat in the period. Negative values of the heat storage, of course, indicate that heat elimination exceeds heat production for the period and the average body temperature has fallen. Alcohol checks with the respiration calorimeter show that it measures the quantity indirect minus direct heat with an average error in hour periods of about 3 per cent. The systematic error is not more than 0.17 per cent, so that the averages of heat storage in a series of experiments may be relied upon.

TABLE 1

Heat storage as percentage of heat produced

DATE 1934	SUBJECT	BASAL		AFTER MEAL				KIND OF MEAL	PER CENT INCREASE IN HEAT PRODUCTION[2]
		1st hour	2nd hour	1st hour	2nd hour	3rd hour	4th hour		
5–2	L.H	— 2.2	— 5.9	(+ 53)[1]	+ 4.1	— 2.6		Meat	20
5–7	L.H	— 5.8	— 7.7	(+ 32)	+ 14.4	— 3.7		C H	24
5–11	J.Ha	— 11.8	— 9.3	(+ 39)	+ 4.9	+ 0.7		C H	24
5–16	G.E	— 12.7	— 2.1	(+ 25)	+ 12.4	+ 7.0		C H	24
5–18	G.Mc	— 21.8	— 23.2	(+ 41)	— 0.8	+ 4.0		C H	31
5–23	K.B	— 21.4	— 11.3	(+ 29)	— 16.9	+ 0.1	— 11.8	Meat	21
6–12	W.		— 10.4	(+ 37)	+ 3.8	— 4.9		C H	16
6–21	J.Hu	— 21.4	— 11.3	(+ 21)	+ 1.2	— 2.1		Meat	22
6–28	R.R	— 7.8	— 7.7	(+ 44)	+ 2.5	— 9.0		Meat	26
	Average	— 13.1	— 9.6	(+ 36)	+ 2.8	— 1.2	(— 11.8)		23

[1] Percentage of basal heat production stored.

[2] Average percentage increase of second and third hours over basal.

Table 1 gives the results of nine experiments. The heat storage, defined above, is given as a percentage of the heat produced by the subject in that hour.

In the basal condition the heat storage in the first hour is invariably negative, the average body temperature evidently falling. The average loss is 13.1 per cent of the heat produced. A review of previous periods in the basal condition (in experiments not related to this study) gives approximately the same figure (— 13.5 per cent). It does not appear that this loss of heat is due to the temperature of the calorimeter chamber (on the average 23.3°C.) being abnormally low, as the loss is not correlated with the environmental tem-

perature at all well. It has been repeatedly observed in this laboratory and elsewhere that the rectal and surface temperatures in basal condition continue to fall steadily for as long as 2 hours when the subject lies down and relaxes completely, even in an environment with a temperature as high as 27°C. The net loss of heat must be considered as the effect of immobilization of the subject, which produces a large decrease in the heat production.

The fact that the average net heat loss in the second of the basal periods is lower than that of the first hour, — 9.6 per cent compared to — 13.1 per cent, is also confirmed in review of previous experiments in the basal condition. In some cases there was an actual storage of heat or a negligible heat loss in the second of two basal periods, in the first of which there had been considerable net loss. This decrease cannot be attributed to any difference in the temperature of the chamber, which was only 0.07°C. higher on the average. Heat production in the two periods was approximately the same, the average for the second period being 1.3 per cent higher than for the first. The bearing of these results on heat loss on the question of heat regulation of the body is discussed later. The magnitude of the discrepancy between heat production and heat elimination in the completely relaxed basal subject emphasizes the need for accurate estimation of heat storage from changes in body temperature if the two are to be brought into agreement (paper II of this series).

For the hour in which the subject was out of the calorimeter and eating the meal, the heat storage can be estimated only from the change that was observed in the body temperatures. It has been calculated using the formula developed in paper II (Burton, '35) for each experiment and is given in the fifth column of table 1 (in brackets). Since the heat production in this period is not known, it is expressed as a percentage of the basal heat production. If expressed as a percentage of the actual heat production in that period it would, of course, be less. There is no doubt that in this period there is a large positive storage of heat. How much is due to the exercise of the subject and how much to the food cannot be stated.

In the succeeding hour, the second hour after the ingestion of food, the heat production tends still to exceed the heat elimination, so that the heat storage is positive. In the next hour the storage becomes slightly negative in the average, the heat production having passed its peak while the heat elimination is still increasing. It is evident that the reactions are slower in some subjects than in others, the positive heat storage occurring in the third hour instead of the second. Presumably this is due to differences in the rates of digestion and absorption. In the single experiment where a third hour is available the body had begun to lose heat once more to a considerable amount.

In only one case can the effects of carbohydrate and protein be compared in the same subject. Here the reaction was evidently more rapid and of a higher peak value with the carbohydrate meal.

The increase in the average rate of heat production in the second and third hours after the meal, over that in the basal periods, is given as a percentage of the latter in the last column. It expresses the usual 'specific dynamic action' of food, in the manner originally used by Rubner and adopted by Lusk.

The observed sequence of heat storage and loss is the consequence of the fact that the extra heat elimination after the meal lags behind the extra heat production, so that there is a temporary rise in body temperature until the increased heat elimination finally compensates. We are studying here the fluctuations and lag of the human thermostat as it meets fluctuations in heat production. In the sections that follow the mechanism of the increased heat elimination is examined more closely.

Water loss. In table 2 the results for the heat lost by evaporation for the same group of experiments are listed. The average percentage of the heat produced that is lost by evaporation is the same for the two basal periods, namely, 17.8 per cent. This figure is considerably less than that usually found, namely, 24 per cent of the heat loss (Soderstrom

and DuBois, '17) although the environmental temperature was in the same range. The same authors found an insignificant decrease to 23 per cent after ingestion of glucose or of protein. In our experiments the decrease in the percentage is quite marked in the second hour after the meal. In the third hour sweating occurred in some of the experiments (shown by the changes in skin temperature and noted by the subject) and the percentage is only slightly less than the basal. The decrease in percentage, expressed in terms of the heat produced, is not an indication that the evaporation loss

TABLE 2

Percentage of heat produced lost by evaporation and as Cals./sq.m./hr. lost by evaporation

DATE 1934	SUBJECT	BASAL				AFTER MEAL					
		1st hour		2nd hour		2nd hour		3rd hour		4th hour	
		Per cent	Cals.	Per cent	Cals.	Per cent	Cals.	Per cent	Cals.	Per cent	Cals.
5–21	L.H	20.0	7.8	20.1	8.3	15.9	7.1	17.5	8.7		
5–7	L.H	16.6	6.6	17.9	7.0	15.2	8.4	16.6	8.4		
5–11	J.Ha	15.1	5.0	16.1	5.5	13.1	5.6	15.4	6.2		
5–16	O.E.	12.9	4.6	12.3	4.8	10.4	4.9	13.1	5.8		
5–18	G.Mc.	18.1	6.4	17.4	6.2	15.8	7.2	18.1	8.5		
5–23	K.B.	17.5	5.4	17.5	5.5	17.4	6.0	16.3	6.5	18.3	6.8
6–12	W.			17.0	6.9	17.7	8.9	20.3[1]	9.0[1]		
6–21	J.Hu	21.0	7.8	21.4	8.1	18.0	8.4	17.7	8.2		
6–28	R.R	21.9	8.3	22.2	8.6	19.8	9.8	22.2[1]	10.4[1]		
Average		17.8	6.5	17.8	6.9	15.9	7.4	17.5	7.9		
Av. percentage of heat elimination		15.8		16.5		16.5		16.5			

[1] Sweating occurred.

decreased after the meal in absolute value. On the contrary, there was a slight increase, as shown by the table. The apparent decrease is due to the fact that the heat lost by evaporation does not increase as rapidly as does the heat production. When expressed as a percentage of the heat lost in the period, the figures are remarkably constant, 15.8 per cent, 16.5 per cent, 16.5 per cent, 16.5 per cent, respectively. This suggests that in this respect the body is losing heat as a purely physical system.

Radiation and convection. Table 3 shows that the burden of the elimination of the extra heat is borne by the increased radiation and convection from the body. There is no doubt about the considerable increase in the hours following the food. Comparison of the averages at the foot of the table for radiation and for heat production brings out the fact, mentioned earlier, that the heat elimination lags behind the heat production. The latter has passed its peak by the third hour, while the former is still rising.

TABLE 3

Radiation and convection in Cals./sq.m./hr.

DATE 1934	SUBJECT	BASAL		AFTER MEAL		
		1st hour	2nd hour	2nd hour	3rd hour	4th hour
5–2	L.H.	31.8	34.6	36.0	42.3	
5–7	L.H.	35.5	35.4	40.1	44.4	
5–11	J.Ha	32.2	31.6	34.9	33.8	
5–16	G.E.	35.5	34.8	36.7	35.7	
5–18	G.Me.	36.6	37.6	38.9	40.9	
5–23	K.B.	31.8	29.4	34.6	33.0	34.5
6–12	W.		38.0	39.4	37.8	
6–21	J.Hu	37.1	33.4	37.8	37.1	
6–28	R.R.	31.7	32.0	37.4	39.6	
Average		34.5	34.1	37.3	38.3	
Average heat produced		36.5	37.4	46.3	45.8	

From physical principles it is clear that the increase in radiation and convection could be brought about only by an increase in the temperature of the surface of the body, the heat originating, of course, from metabolism.

Temperature changes. The skin temperature thermometers measure directly the average temperature over portions of the surface of the body, which are in area about 930 sq.cm. for the trunk, 600 sq.cm. for the lower leg, and 360 sq.cm. for the forearm. Care was taken that the conditions of clothing worn over these thermometers should be identical throughout the experiment, so that the observed changes might be of physiological significance. The temperatures showed considerable

consistency, the fluctuations, as distinguished from systematic trends, rarely exceeding 0.1°C. from one observation to the next, 5 minutes later. The occurrence of sweating could be seen at once in the sudden drop of temperature and the subsequently increased fluctuations. Muscular movements, as those of the arm in operating the signaling device in the chamber, raised the temperature momentarily. In the averages taken over the whole period, a change of skin temperature of more than 0.1°C. can be considered significant.

In the basal period the rectal temperature continued to fall for almost the whole of the first 2 hours that the subject was lying down, remaining, on the average, constant or even ris-

TABLE 4

Average temperature changes in °C.

PLACE	BASAL		AFTER MEAL		
	1st hour	2nd hour	1st hour	2nd hour	3rd hour
Rectal	−0.09	+0.02	+0.26	+0.02	0.00
On trunk	−0.06	−0.15	+0.78	+0.25	−0.12
On lower leg	−0.23	−0.39	+0.93	+0.09	0.00
On forearm	−0.01	−0.21	+0.80	+0.08	−0.16
Average surface	−0.08	−0.25	+0.79	+0.18	−0.08

ing in the third hour. The surface temperatures, however, falling with the rectal at about the same rate for the first hour, fell much more rapidly in the second and third hours. This is brought out by the averages for all the experiments in table 4. (The average surface temperature is calculated from 0.5 × trunk plus 0.36 × leg plus 0.14 × arm temperatures, a formula weighting the temperatures according to the mass of tissue beneath them.) The fall is most marked on the leg, least on the arm. The total fall of surface temperature is much greater than that of rectal temperature. In one female subject, the temperature on the surface of the leg fell a total of 3.4°C. in the 3 hours, from 31.5° to 28.1°C., while the rectal fell only 0.25°C.

These changes of temperature give evidence of the operation of the heat regulating mechanism of the body against loss of temperature. In the first hour the body cools as a purely physical system, the surface falling an amount approximately equal to the fall of interior temperature. In the following hours, however, the surface falls a great deal more than the interior, indicating a decrease in the heat conductivity of the peripheral tissues, due to a vasoconstriction and decreased

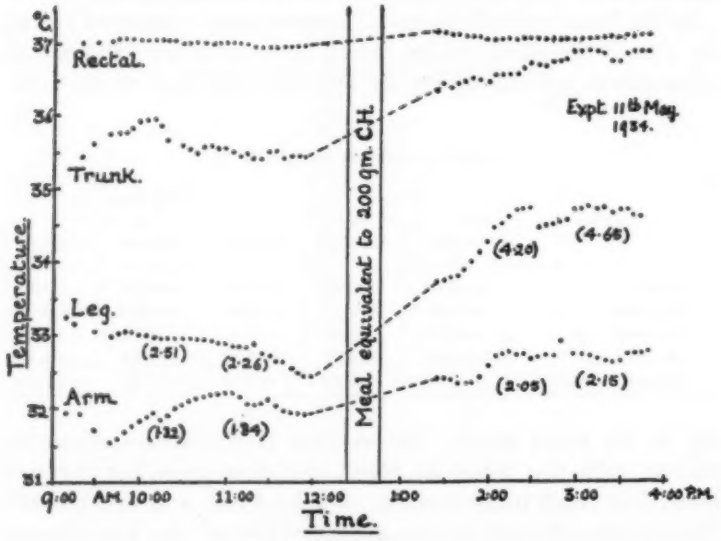

Figure 1

blood flow. That this physiological adjustment is effective is proved by the cessation of the fall of rectal temperature and by the decrease in the net heat loss (table 1).

In the hour immediately following the meal the rectal temperature is the first to rise, remaining on the average steady in the next 2 hours. The surface temperatures show a very much greater increase, which continues in the second hour, falling finally in the third. In figure 1 the records of temperature of one experiment are plotted to show the time rela-

tions of the changes. Without exception in the experiments, the surface temperatures were maintained at a much higher level after the meal, even though in some cases the rectal temperature had fallen. The average levels of the surface temperatures during the hour periods are given in table 5.

The rise of temperature is usually the greatest for the surface of the lower leg, least for the forearm (as explained later, however, this does not mean that the change in blood flow is greatest in the legs).

Effects of exercise. It is obvious that in the periods following the meal, the effects of immobilization of the subject are superimposed upon those due to the ingestion of the food.

TABLE 5

Average level of temperatures in °C.

PLACE	BASAL		AFTER MEAL	
	1st hour	2nd hour	1st hour	2nd hour
Rectal	36.72	36.68	36.92	36.96
Trunk	34.52	34.36	35.13	35.07
Lower leg	32.16	31.78	32.60	32.84
Lower arm	31.32	31.09	31.75	31.65
Calorimeter chamber	23.30	23.37	23.40	23.59

Since, however, exercise preceded the basal periods just as it did the periods following the meal, a comparison of the changes of temperature in corresponding periods tends to eliminate this factor. In order that the time relations of the temperature changes due to the food might be examined unaccompanied by the effects of exercise, an experiment was made in which the subject lay in the calorimeter in basal condition for about 2 hours, the second of which was a calorimetry period. He then, at a signal, ate a meat meal while lying there, making as few muscular movements as possible, and two calorimetry periods of an hour each followed.

There was an immediate small rise of rectal temperature of about 0.08°C. and of about 0.4°C. in the temperature on the trunk after the meal was finished, but this proved temporary

only. No simultaneous rise was recorded in the temperatures on the leg or arm. About 1½ hours later, however, a significant and rapid rise in surface temperatures was evident. As the subject had been in the calorimeter for 4 hours, the rise could be followed for some 20 minutes only. The plausible explanation of this delayed rise is that in this experiment the emptying of the stomach and absorption had been delayed, and that as Herrick and co-workers ('34) found in their experiments on dogs, the increased blood flow does not occur unless there is absorption. The value of heat production in the 3 hours bears out this conclusion. It was 78.9 Cals./hr. in the first, basal hour, 89.3 in the hour during which there was exercise, and 82.8 in the last hour during complete quiescence. The specific dynamic action had only just started when the experiment ended. A second experiment of this character failed, because of the inability of the subject to ingest the meal while in the prone position.

Two experiments were made in which the surface and rectal temperatures were taken on a subject lying on a bed in a cloth covered cubicle, the walls of which contained resistance wire thermometers. No metabolism studies were made in order that disturbing effects due to lack of relaxation of the subject might be eliminated. The meal, 200 gm. of lean chopped beef, was eaten by the subject still on the bed, but with the head and shoulders propped up. It is thought that these experiments give a true picture of the effects of food ingestion, with the least complication due to muscular exercise. One of them is illustrated in figure 2. A control experiment with similar conditions, but no ingestion of food is shown in the same charts. Comparison enables the time relations of the temperature changes to be estimated fairly accurately. There was an immediate rise in rectal temperature, and a very rapid rise of the temperature of the leg, even though this had not been moved at all. The apparent cooling of the arm after the meal is seen to be the normal fall in the basal condition until the rise began some 30 minutes after the meal was finished. The maximum was reached at about 1½ hours after ingestion.

The data for another experiment, in which the metabolism was taken in ten minute periods every hour by the Tissot-Haldane method, are given in table 6. The periods were of

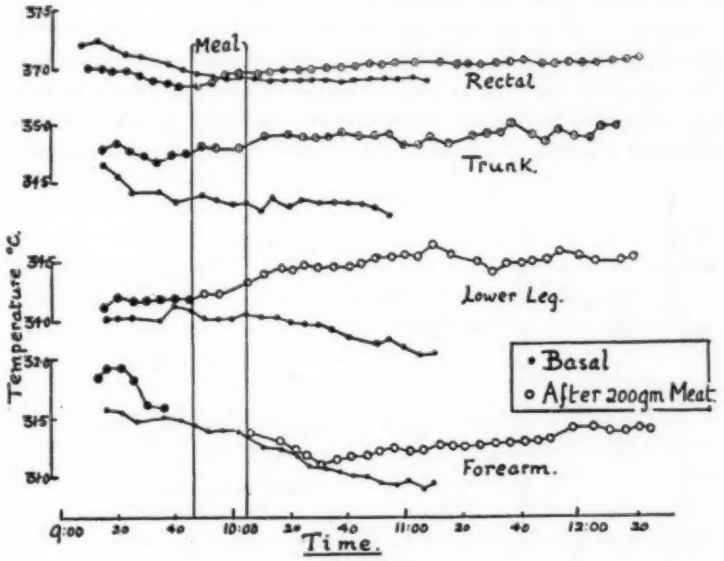

Figure 2

TABLE 6

Period	I	Meal	II	III	IV
R.Q.	.82		.80	.80	.80
Metabolism Cals./hr.	65.5		73.0	79.3	82.8
Increase over period I			11.4%	21.2%	26.4%
Average rectal temperature, °C.	36.97		37.08	37.09	37.13
Increase over period I, °C.			.11	.12	.15
Average temperature on trunk, °C.	34.77		34.93	35.40	35.44
Increase over period I, °C.			.16	.63	.67
Average temperature on leg, °C.	32.55		33.13	33.74	33.38
Increase over period I, °C.			.58	1.19	.83
Circulation index trunk	4.02 (100)		4.01 (100)	5.43 (135)	5.49 (137)
Circulation index leg	1.50 (100)		1.72 (113)	2.24 (149)	1.92 (128)

1 hour each, and the meal was eaten at the start of the second hour. The results, so far as temperature changes go, are comparable with those shown in the charts. The last lines of the table show the thermal circulation index.

Finally, two experiments were made with the subjects lying unclothed in a room of constant temperature and humidity.[1] The changes in the average temperatures on the abdomen are shown in figure 3. The changes on the leg and forearm followed a similar course. The drop in temperature immedi-

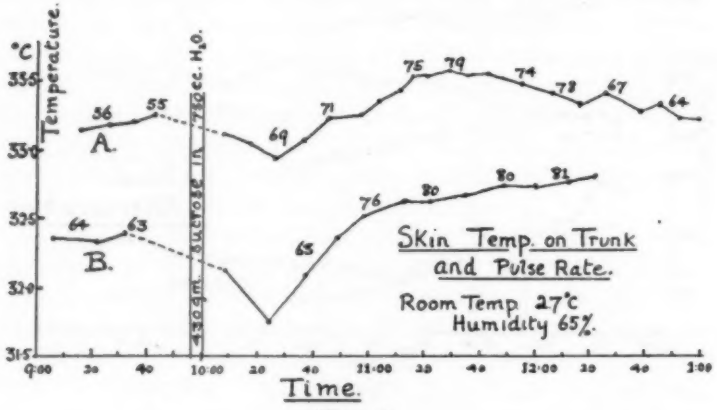

Figure 3

ately following the ingestion is due to the fact that the solution was at low temperature (about 15°C.), and corresponds well in amount with that calculated to supply heat sufficient to raise the weight of fluid to body temperature. The difference in the time relations of the reactions is marked, and corresponds with the fact, known from previous experiments, that absorption and diuresis are slower in subject B than in A. There was definite glycosuria in A, but none detectible in B after the sucrose ingestion.

[1] Coincident studies of insensible loss in short intervals by weighing and of urine excretion were carried on by Mr. G. McClure and Mr. J. Hall, for whose courtesy in using the data we are indebted.

The whole group of experiments leaves no doubt that there is a significant rise of surface temperature of the human body much greater than the coincident rise of rectal temperature, after the ingestion of solid or liquid carbohydrate or protein meals. The failure of Herrick and co-workers (loc. cit.) to find any significant rise of temperature on the forearm of a human subject or the leg of a dog must be attributed to the insensitiveness of their apparatus or lack of control of environmental conditions. It is interesting to note that Williams, Riche and Lusk ('12) recorded that there was a rise in skin temperature of a dog after ingestion of food greatly exceeding the rise of rectal temperature.

Blood circulation. The fact that the changes in surface temperature both in the basal and absorptive periods, exceed the changes in internal temperature, means that there have been changes in circulation of blood, since, in a purely physical system where the properties of heat transfer are constant, the changes in surface temperature must be less than those of the interior. This is expressed quantitatively by the use of the 'thermal circulation index' (Burton, '34 b).

$$\text{T. C. I} = \frac{\text{External drop of temperature}}{\text{Internal drop of temperature}} = \frac{\text{Surface temp.—Room temp.}}{\text{Rectal temp.—surface temp.}} = \frac{C}{C^1}$$

The index compares the coefficient of heat transfer, for the tissues, C^1 with that for the environment, C. An increase in the index for a given surface area is brought about by increased local or general circulation, a decrease by the reverse. Constancy of the index means that the body is behaving as would a purely physical system of constant thermal properties.

A correction to the simple formula is required if the evaporation from the body changes greatly. In the case of the calorimetry experiments, the results of which are listed in tables 2 to 5, this correction may be made with accuracy. We have, for the heat lost by radiation and convection from an area of surface (surface temp. T_s, environmental temp. T_e)

$$H_R = C\,(T_s - T_e).$$

and for the total heat brought up to that area of surface—

$$H = C' (T_1 - T_s)$$

Then, by division—

$$T.C.I = \frac{C'}{C} = \frac{T_s - T_t}{T_1 - T_s} \times \frac{H}{H_s}$$

The correction factor $\frac{H}{H_s}$ may be obtained from tables 2 and 3, if we assume that for the particular area in question it is equal to the ratio of the total heat eliminated in the period to the total heat lost by radiation and convection in that period.

TABLE 7

Thermal circulation indices

PLACE	BASAL		AFTER MEAL	
	1st hour	2nd hour	1st hour	2nd hour
Trunk	6.66 (100)	5.70 (86)	7.87 (118)	7.32 (110)
Lower arm	2.30 (100)	2.07 (90)	2.55 (111)	2.73 (119)
Forearm	1.76 (100)	1.66 (94)	1.94 (110)	1.83 (104)

The correction in the case of the average temperatures of table 5 proves to be insignificant, the factors for the four periods being 1.18, 1.20, 1.20 and 1.20, respectively. The corrected indices are given in table 7. For comparison they are reduced so that the figure for the first basal period is 100 (in brackets).

The index is significantly greater after the meal than in the corresponding basal periods, by from 10 to 27 per cent. In individual experiments the increases in the indices were often of much greater amount, from 30 to 50 per cent, the averaging of experiments with differing time relations leading to lower figures. The figures for the experiment of table 6 are given at the foot of that table, and indices are also given (in brackets) on the chart (fig. 1). The use of the T. C. I.

shows that though the changes in temperature on the leg are greater, those on the trunk are just as significant as an indication of increased blood flow. The reaction in the arm seems of smaller magnitude. There is no indication whatever of any decrease of blood flow to the peripheral tissues, but very definitely to the contrary, there is a general increase of circulation after ingestion of food.

Pulse rate. In some of the calorimeter experiments and in those illustrated in figure 3, the pulse rate was taken at frequent intervals. In the basal condition there was only a slight tendency for the rate to decrease after the first few minutes, but after the meal there was a marked increase. That this is closely correlated with the increase of surface temperature is shown in figure 3. The increased peripheral blood flow indicated by the rise of T. C. I. need not then be ascribed to any vasomotor shift, but is explained (as in the dog by Herrick and co-workers) by a general increase in blood flow throughout the body. The cooling of the surface in the later basal periods is more likely due to a peripheral vasoconstriction.

CONCLUSIONS

The inferences from the experiments have been discussed under the various headings, but may be illustrated by figure 4, which shows the results of a fictitious experiment deduced from the average values of all the experiments. The chart is self-explanatory. The dotted area of the blocks shows the net storage or loss of heat in the hour periods. If the full line (heat production) is above the dotted line (heat elimination) the storage is positive, and vice versa.

The stimulation of the heart rate and consequent rise of the surface temperature more rapidly than the interior may well be coincident with the rise of heat production (S. D. A). The thermal capacity of the body, however, would make the rise of temperature lag behind the heat production and persist some time after the latter had reached its peak. We have, at present, no evidence that contradicts the assumption that the circulatory and thermal reactions are stimulated by the same

factors as the specific dynamic action. Careful comparison of the effects of protein and carbohydrate may later provide such evidence.

The supposed vasomotor shift of blood from the periphery to the viscera during absorption is seen to be contradicted by the facts. Such a shift would lead to a cooling of the body surface instead of a rise, and some other mechanism, such as

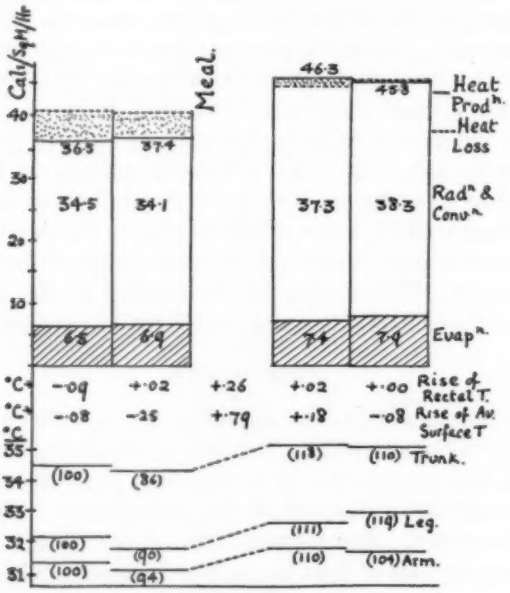

Figure 4

increased sweating, would have to be called upon to bring about elimination of the extra heat produced and prevent excessive rise of body temperature. The actual mechanism of a general increase of blood flow throughout the body serves the double purpose of supplying the viscera with the additional blood required by absorption and also of eliminating the extra heat produced.

SUMMARY

The heat production, heat elimination, and the rectal and surface temperature changes of ten adult subjects have been studied in the respiration calorimeter, in basal condition and after the ingestion of carbohydrate and protein meals.

On the average, heat elimination exceeds heat production by 13 per cent in the first basal hour and 9 per cent in the second. The body first falls in temperature uniformly; later, the surface falls much more than the rectal temperature, indicating a heat regulating adjustment.

After the meal heat is first stored in the body by general rise of temperature, but later the surface temperature rises much more than the rectal temperature (about 1°C. compared to 0.25°C.), radiation from the body is increased and the extra heat is eliminated. Increased evaporation plays a minor role. The rise of surface temperature is most marked on the leg, but the use of the 'thermal circulation index' shows the increased circulation is general. It commences 20 minutes after the ingestion. The effects of protein and of solid and liquid carbohydrate are similar. They are closely correlated with an increase pulse rate after the meal.

There is no evidence of a withdrawal of blood from the somatic tissues to the visceral, but a definite general increase in circulation, which may well be due to the same factors as cause the specific dynamic action.

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THE PHYTIN PHOSPHORUS OF THE CORN COMPONENT OF A RACHITOGENIC DIET[1]

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ONE FIGURE

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It has long been assumed that the three dietary factors of importance in the production of experimental rickets in rats are low content of vitamin D, low percentage of phosphorus in respect to the total diet (0.25 per cent or less) (Brown et al., '32), and a low proportion of phosphorus to calcium (not more than 1:4) (Steenbock and Black, '25). That there are other factors of importance and that the classical concepts just stated are in error can now be safely asserted. The early observations of E. Mellanby ('21) on the anti-calcifying properties of oatmeal are not explained by the three primary criteria for rachitogenic diets; recent work of Kon and Booth ('34) indicates an anti-rachitic effect of the saponifiable fraction of butter fat; diets compounded and used in our own laboratory, in work to be reported later, which were devoid of extractable vitamin D, low in total phosphorus, and with Ca:P ratios as exaggerated as 8:1, have failed to induce rickets.

In a previous contribution (Harris and Bunker, '34) we reported variations in the rachitogenic properties of forty samples of corn (maize) compounded into batches of the Steenbock no. 2965 rachitogenic ration (Steenbock and Black, '25),

[1] Contribution no. 47 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Massachusetts.

all ingredients other than corn being identical to all lots of diet. Data were supplied to show that these variations were due not to differences in amount of total phosphorus, nor to the Ca : P ratios of the various batches of diet, but were associated with some variations residual in the corns themselves. We suggested that in the corn component there might be linkages of phosphorus with other chemical elements constituting compounds which vary among each other in respect to their assimilability and in respect to their relative proportions in different samples.

The establishment of the classical Ca : P = 4 : 1 ratio of a rachitogenic diet appears to be the result of determinations of the total calcium and total phosphorus in diets which previously had empirically proved to be rachitogenic. It is no discredit to the original investigators in this field that what appeared to be a causative correlation between calcium-phosphorus ratios and the development of experimental rickets now is perceived to be a relation applying only to certain limited conditions, as for instance, those obtaining in a rachitogenic formula comprising a large proportion (76 per cent) of corn.

If the hypothesis be valid that one or more forms of phosphorus which contribute to the sum of total phosphorus, as usually determined by drastic chemical methods, is of particular metabolic significance to the rat, then we have at least a hypothetical explanation of the failure of the classical total calcium-total phosphorus ratio to support rickets in certain experimental diets. Support of this hypothesis is drawn by us from the papers of Lecoq and Villette ('33) and of Bruce and Callow ('34) in which the addition to diets of phosphorus in various forms (mostly inorganic) has received attention from the point of view of its effect on healing of rickets. If variations in the form of phosphorus administered produce different effects upon the metabolism of healing, there may well be analogous effects upon the metabolism associated with the causation of rickets varying with the type of phosphorus fed. The really significant criteria concerning the phos-

phorus content of a rachitogenic diet may be restricted to the quantities or proportions of certain types rather than to the total phosphorus values.

The present contribution is based on the first of our studies of the significance of the several fractions of rachitogenic diets which contain phosphorus in one form or another, and deals specifically with the phosphorus of the phytin fraction of corn. Phytin was selected as obviously being quite different in its relation to the total phosphorus of animal and vegetable dietary ingredients, and because we have observed that a rachitogenic diet can be compounded more successfully from corn, for instance, than from casein. Phytin accounts for a large proportion of the total phosphorus of corn; it is absent from casein.

We have estimated the amount of phytin P in each of the forty corns which were the subject of the previous study, using our modification (Harris and Mosher, '34) of the Heubner-Stadler method. The results are given in table 1, column 5. The determinations of the total phosphorus of these corns (column 2) and the animal experiments were carried out at the same time. The quantitations of the phytin phosphorus were made several months later.

The only other source of phosphorus in the diet no. 2965 is wheat gluten, which comprises 20 per cent of the ration. In the diet as fed, the gluten contributed 0.068 per cent total phosphorus and 0.037 per cent phytin phosphorus. As the gluten used in the animal experiments was the same in all cases, the phosphorus fractions contributed by it did not contribute to the variations with which we are concerned.

The purpose of this study was to find whether a correlation exists between the phytin phosphorus content of corn and the degree of rickets it produces when incorporated in the no. 2965 Steenbock ration. To assist visualization of this relationship the total phosphorus and the phytin phosphorus content of the corn in each of the forty rations have been plotted against the degree of rickets each ration produced (fig. 1). There is no apparent correlation.

TABLE 1

CORN NO.	BREED AND SOURCE	1932 ANALYSES			1934 ANALYSES			PER CENT CORN P IN NO. 2965 DIET AS FED [1]	
		Per cent moisture	Per cent total P		Per cent moisture	Per cent phytin P			
			Wet	Dry		Wet	Dry	Total	Phytin
1	Haskins yellow, Mass.	8.4	0.285	0.312	7.4	0.229	0.247	0.218	0.172
2	Golden Glow Dent, Mass.	8.2	0.351	0.382	8.4	0.253	0.276	0.266	0.193
3	Lancaster Sure Crop, N. J.	12.1	0.324	0.368	8.6	0.240	0.262	0.246	0.175
4	Clarage, Ohio	8.4	0.301	0.332	8.8	0.250	0.273	0.232	0.190
5	Lancaster Sure Crop, Del.	14.0	0.289	0.336	10.4	0.135	0.150	0.220	0.098
6	Minn. 13 (Sd. 86), S. Dak.	8.0	0.370	0.400	8.0	0.315	0.342	0.280	0.239
7	Williams Yellow Dent, Mass.	8.3	0.333	0.364	8.1	0.241	0.262	0.254	0.182
8	Minn. 13, Thorpe, N. Dak.	11.5	0.342	0.396	8.2	0.265	0.289	0.266	0.195
9	Duncan Yellow Dent, Mich.	11.4	0.356	0.400	9.0	0.245	0.269	0.269	0.181
10	Not Known, Wis.	9.0	0.326	0.359	9.6	0.247	0.273	0.248	0.189
11	Thatcher's Gold Dent, Ark.	7.9	0.295	0.320	9.3	0.249	0.274	0.224	0.192
12	Not Known, Ill.	13.7	0.293	0.340	8.4	0.230	0.251	0.223	0.164
13	Longfellow-Davis Flint, Mass.	9.3	0.273	0.300	11.5	0.194	0.219	0.207	0.151
14	Not Known, Ill.	12.4	0.314	0.358	9.2	0.279	0.307	0.238	0.204
15	Hulsart Yellow Dent, N. J.	13.3	0.351	0.404	8.9	0.273	0.299	0.266	0.197
16	Kruger, Ill.	12.3	0.326	0.372	8.3	0.190	0.207	0.248	0.138
17	Golden Glow, Mich.	11.6	0.313	0.354	8.2	0.250	0.272	0.238	0.182
18	Yellow Dent seed corn, Utah	12.2	0.376	0.427	8.3	0.293	0.319	0.284	0.213
19	Sure Crop, Penn.	8.0	0.317	0.345	8.1	0.243	0.264	0.242	0.185
20	Hall's Gold Nugget Flint, N. J.	12.7	0.355	0.407	8.3	0.248	0.270	0.270	0.179
21	Polar Dent, Mich.	11.6	0.381	0.432	7.9	0.271	0.294	0.290	0.198
22	St. Paul 13 Dent, Minn.	12.0	0.364	0.414	8.2	0.280	0.305	0.277	0.204
23	MAC Yellow Dent, Mich.	11.8	0.326	0.370	7.9	0.224	0.243	0.248	0.163
24	Reid's Yellow Dent, Neb.	13.8	0.335	0.389	8.7	0.230	0.252	0.255	0.165
25	Golden Queen, Penn.	8.2	0.312	0.340	7.8	0.267	0.290	0.238	0.202
26	Western Plowman, Ill.	12.9	0.306	0.351	8.1	0.223	0.243	0.232	0.161
27	Reid's Yellow Dent, Del.	13.6	0.275	0.318	9.6	0.136	0.150	0.209	0.099
28	Reid's Yellow Dent, N. J.	12.8	0.332	0.381	10.0	0.243	0.270	0.252	0.179
29	Not Known, Kan.	8.4	0.343	0.375	8.0	0.261	0.284	0.261	0.198
30	Wilson Yellow Dent, Fla.	12.2	0.355	0.404	8.5	0.252	0.274	0.270	0.183
31	Somerset Teaming Dent, N. J.	12.9	0.279	0.320	11.8	0.202	0.229	0.212	0.152
32	Cattle, Neb.	14.0	0.372	0.435	9.4	0.245	0.270	0.284	0.176
33	King Philip Hybrid, Calif.	12.4	0.354	0.404	7.9	0.231	0.251	0.269	0.167
34	Pickett Yellow Dent, Mich.	11.7	0.300	0.340	8.7	0.239	0.262	0.228	0.176
35	Reid's White Cap, N. J.	12.0	0.337	0.383	7.4	0.289	0.312	0.256	0.208
36	Mercer Flint, N. Dak.	11.2	0.332	0.374	7.9	0.227	0.247	0.252	0.166
37	Mercer White Cap Yellow Dent, N. J.	12.2	0.300	0.342	11.2	0.251	0.284	0.228	0.189
38	Clement's White Cap, Mich.	12.1	0.300	0.341	8.9	0.223	0.245	0.228	0.163
39	White Flint, R. I.	12.9	0.364	0.418	7.4	0.278	0.300	0.277	0.198
40	Moore's White, Wis.	12.5	0.296	0.338	15.0	0.195	0.229	0.225	0.152
	Average	11.1		0.369	8.9				
	Column number	1	2	3	4	5	6	7	8

[1] On basis of wet weight in 1932.

DISCUSSION

A decade or more ago, E. Mellanby ('21) noted that cereals are rachitogenic (anticalcifying) in spite of their relatively high total phosphorus content. He could not find that the anticalcifying effect is correlated with the fat, carbohydrate, relative or absolute amounts of calcium and phosphorus, nor acid-base relations of their rations. Steenbock et al. ('30) re-

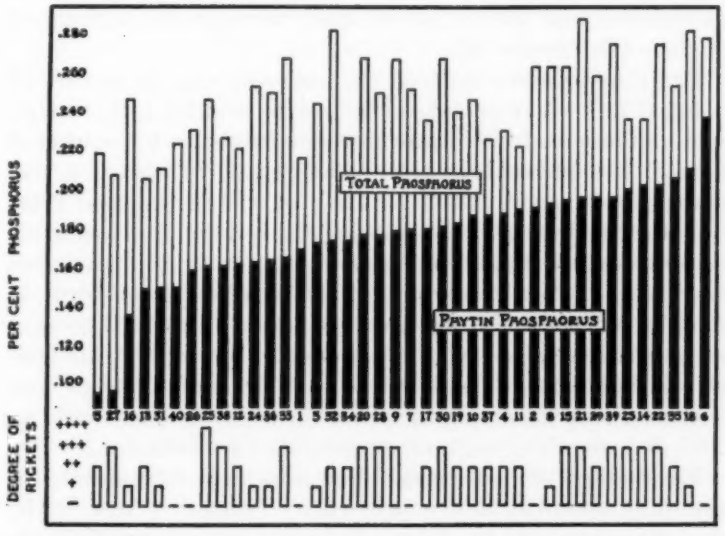

Fig. 1 Relation of corn phytin phosphorus in the diet as fed to the degree of rickets produced.

ported more recently that the calcifying property of certain grains, supplemented by calcium carbonate, could be arranged in the descending order, wheat, oats, corn, and that the total phosphorus of these grains is in the reverse order. These findings and others have been discussed by Bruce and Callow ('34) in a report which has appeared while our own data were being analyzed. They advance the view that inositol hexaphosphoric acid (phytic acid) may be the elusive factor in these cereal diets, exerting non-calcifying effect because of

its low assimilability. For the sake of accuracy, it should be pointed out that inositol hexaphosphoric acid occurs in grains in the form of phytin, a salt of the hexaphosphoric ester of inositol (Posternak, '19) and that associated with this substance there occurs naturally the enzyme phytase which under suitable moisture and temperature conditions catalyzes the hydrolysis of phytin, yielding inorganic salts of orthophosphoric acid. In sprouting grains phytin is split to inorganic phosphates and inosite, presumably through the activity of phytase (DeTurck et al., '33).

Bruce and Callow reduced the anticalcifying properties of oatmeal and other cereals by a treatment with hydrochloric acid and assumed the improvement to be due to hydrolysis of inositol hexaphosphoric acid. They state, in regard to the unexplained variations in severity of rickets induced from time to time by a rachitogenic diet, in spite of its reasonably careful control, that "one possible factor in this variation may be the proportions of phytin and other phosphorus in samples of maize of similar phosphorus content," a view which we entertained at the beginning of our work. Analysis of our data fails to demonstrate that either absolute or proportional amount of phytin phosphorus in the Steenbock no. 2965 diet correlates with the degree of rickets obtained.

The findings of Bruce and Callow appear to substantiate the generally accepted view expressed by Plimmer ('13 a, '13 b) that phosphorus in the form of phytin is poorly available in the nutrition of carnivores and herbivores. An important part of the evidence of these authors is based on the finding that acid treated oatmeal shows an apparent greater availability of phosphorus (at least, less anticalcifying tendency) than the untreated cereal, and the assumption is natural that this improvement is related to the acid hydrolysis of phytin with a resultant increase in the proportion of inorganic phosphates which, under suitable conditions, have been shown by many workers to be available for calcification processes.

The finding of Templin and Steenbock ('33 a, '33 b) that germinated corn is rendered definitely less rachitogenic by

autolysis, during which process it may be assumed that phytin phosphorus is actively hydrolyzed, is concordant with the above view of Plimmer. A previous report by these authors that unripe maize is less rachitogenic than the fully ripened grain is also suggestive, since DeTurck ('33) has demonstrated that phytin is formed at or near the point of storage (of phosphorus) in the ripening of seeds. He also quotes Zaleski in Kostychev's 'Plant physiology' ('31) as pointing out that during the ripening of seeds, a considerable amount of phosphorus enters the seed in the form of . . . . phytin. Quantitative data on the relative proportions of phytin and non-phytin phosphorus in germinated and hydrolyzed grains seem to be necessary in order that their real bearing upon the general question of availability of phosphorus may be established.

It would appear at first glance that the work reported by us previously on the increase of rachitogenic properties of corn stored after grinding in loosely covered containers for a period of months is not in accord with the theory that phytin phosphorus is less available than the inorganic derivatives of its hydrolysis. It would seem that storage might permit phytase action through which the availability of phosphorus would be increased and rachitogenic property decreased. The relatively dry state of our corns during storage permitted relatively feeble phytase action, although not prohibiting oxidation of classical vitamin D. One may presume that any decrease in the rachitogenicity of stored corn, attributable to a reduction of phytin, is outweighed by other changes, the net result of which is the observed increase in rachitogenic effect. The apparent discrepancy between our findings and the theory of relative non-availability of phytin then disappears.

While it is important to consider the matter of phytin phosphorus in materials used for compounding a rachitogenic diet, our results strengthen our opinion that too much emphasis must not be laid upon this factor alone; that there are other factors contributing to the effects of such diets which may

completely overshadow the alleged phytin effect. Perhaps undue attention is being paid to the matter of phosphorus in such diets, but it is necessary to evaluate this factor as well as others before the real dietary criteria of rachitogenicity can be established. A continuation of an investigation of other groups of phosphorus in corn is proposed in order that any demonstrable differences in their availability in the rachitogenic diet may be determined.

CONCLUSIONS

The variations in rachitogenic properties of forty corn samples used in the Steenbock no. 2965 ration do not correlate with the absolute or relative amounts of phytin phosphorus, nor with the 'non-phytin phosphorus' fraction of these corns, nor with the total Ca : total P ratio of rations as fed.

It has been shown that the content of inositol hexaphosphoric acid (salts) is not the sole cause of the anticalcifying action of all cereals. In the case of corn, at least, there exists some other important factor or factors.

Until the unidentified rachitogenic factors of corn are understood and controllable, the incautious use of this ingredient in a 'standardized' rachitogenic diet may be expected to produce discordant results in the bio-assay of vitamin D.

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PRODUCTION AND COMPOSITION OF SOW'S MILK

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(Received for publication August 11, 1934)

Information concerning milk production of the sow, the composition of sow's milk, and the constituents of its ash is limited. A more complete knowledge of sow's milk was needed, to give a fuller understanding of the nutritional requirements of the fast-growing pig and to aid in formulating suitable rations. The available information has been collected and to it has been added data obtained at this station.

MILK PRODUCTION

Production records of sixty sows and seventy-six lactations are presented in table 1. The average daily and, where possible, total production for one lactation are shown. The sows from which the records were obtained varied in age and weighed from 250 to 500 pounds each.

These data are reported for the years 1865 and 1933, inclusive. As would be expected, there are wide variations in the average daily and total milk production. The lowest production was obtained by von Gohren (1865) and the highest by Ostertag and Zuntz ('08). The daily production reported by von Gohren (1865) is far below the average and is given for its historical interest. The average daily production for seventy-five lactations was 6.8 pounds. Henry and Woll (1897) reported the milk yield was not large immediately after farrowing and at weaning time—that the maximum flow was in the middle of lactation; Davies ('04) found a gradual increase in production to the sixth week after parturition and

completely overshadow the alleged phytin effect. Perhaps undue attention is being paid to the matter of phosphorus in such diets, but it is necessary to evaluate this factor as well as others before the real dietary criteria of rachitogenicity can be established. A continuation of an investigation of other groups of phosphorus in corn is proposed in order that any demonstrable differences in their availability in the rachitogenic diet may be determined.

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MILK PRODUCTION

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These data are reported for the years 1865 and 1933, inclusive. As would be expected, there are wide variations in the average daily and total milk production. The lowest production was obtained by von Gohren (1865) and the highest by Ostertag and Zuntz ('08). The daily production reported by von Gohren (1865) is far below the average and is given for its historical interest. The average daily production for seventy-five lactations was 6.8 pounds. Henry and Woll (1897) reported the milk yield was not large immediately after farrowing and at weaning time—that the maximum flow was in the middle of lactation; Davies ('04) found a gradual increase in production to the sixth week after parturition and

a gradual decrease thereafter; and Carlyle ('03) states that some sows yield nearly double that of others. Schmidt and Lauprecht ('26) report that sows fed a high-protein diet produce more milk than those on a low-protein ration; that sows suckling 8.5 or more pigs yield more than those having fewer pigs, and that sows suckling their third to sixth litters pro-

TABLE 1

Milk production of sows

NUMBER OF SOWS	AVERAGE NUMBER OF PIGS IN LITTER	INDIVIDUAL DAILY PRODUCTION	PRODUCTION DURING LACTATION			REPORTED BY
			8 weeks duration	10 weeks duration	12 weeks duration	
		pounds	*pounds*	*pounds*	*pounds*	
1	...	3.4	....	....	....	von Gohren (1865)
4	6.8	5.2	....	364.0[3]	....	Henry and Woll (1897)
12	7.2	5.4	....	....	464.9	Carlyle ('03)
1	7.0	5.6	....	389.2	....	Davies ('04)
3	...	11.4	638.4	....	....	Ostertag and Zuntz ('08)
1	9.0	10.2	....	....	....	Schmidt and Lauprecht ('26)
1	7.0	11.6	....	....	....	
1	9.0	7.2	404.9	....	....	
1	8.0	8.2	460.5	....	....	
1	6.0	10.7	600.0	....	....	
20[1]	8.3	6.9	388.2	....	....	
4	...	6.9	....	481.0	....	Ohligmacher[3]
7	...	6.5	....	457.7	....	Ohligmacher[3]
2	7.5	7.7	....	539.0	....	The authors
Average	*7.9*	*6.8*	*413.2*	*447.3*	*464.9*	

[1] Thirty-six lactations.
[2] Calculated from daily production.
[3] Reported by Schmidt and Lauprecht ('26).

duce more milk than sows in their first and second lactations. They found, too, that sows with a higher protein diet gave much more milk in the middle of lactation than those getting less protein; that older sows produce more milk in the early part of lactation, whereas the young sows gave the same amount in the late as in the first part of the period.

The authors found, for the two sows tested, their maximum production was attained between the second and fourth weeks

of lactation and that their total production was nearly the same. In 10 weeks one sow produced 553 pounds and the other 525 pounds of milk. These figures are above the average of other production records reported.

METHODS OF OBTAINING SAMPLES FOR ANALYSIS AND TOTAL YIELD

Some difficulty was experienced in obtaining milk samples. Little success was realized until the milk-secreting habit of the sow was understood. It was found that she does not give her milk 'down' continuously while the pigs are suckling, but there seemed to be a definite succession of events in the process. The sow may allow the pigs to suckle for some time without giving milk. After sufficient stimulation by the rooting, squealing pigs she begins grunting in a characteristic manner, easily understood and recognized by an experienced operator. Suddenly the milk starts coming and flows freely. The pigs cease their rooting and clamoring, settle back and suckle. This continues for about 60 seconds when the milk flow ceases as suddenly as it began.

Considering these facts, the operator must govern himself accordingly. He proceeds to the sow quietly so that she is not disturbed. When the milk flow begins he must work quickly, keeping a pig away from the teat from which the sample is being taken. Samples of 30 to 50 cc. are often obtained from a single gland.

Samples of colostrum are more readily obtained than normal milk. During parturition and before the newborn pigs suckle, the operator proceeds quietly to the sow and obtains a sample in a similar manner as one would milk a cow. Pressure exerted approximately in the center of the mammary gland system seems to aid in making the colostrum flow more freely. After the samples were drawn they were immediately placed in an ice box and kept for analysis. Attempts were made to obtain samples by using a breast pump and a small milking machine with poor success.

The total production of a sow was obtained by allowing the pigs to suckle at definite intervals of time for a 24-hour period. The pigs were weighed before and after suckling and the increase in weight was taken as a measure of the milk secreted for that period. This was done every 10 days and was considered the average yield for the 10 days. In the early stages of lactation the pigs were allowed to suckle every 2 hours; later during the production test each 4 hours; and near the end of lactation the intervals were increased to 6 hours each.

METHODS OF ANALYSIS

Total solids were obtained by evaporation on a steam bath and then drying in an oven at about 90°C.; ash was determined by ashing the dried sample in an electric muffle at about 500°C. More difficulty was experienced in ashing colostrum than normal milk. Calcium was determined by dissolving the ash in dilute HCl and transferring to a volumetric flask. Various sizes of volumetric flasks were used, according to the weight of the ash. Roughly, the desired concentration was about 0.04 gm. ash in a 50 cc. volumetric flask. An aliquot of 2 cc. was taken and put into a 15 cc. centrifuge tube. To be sure the solution was at the correct pH, brom cresol purple was used as an indicator. From this point the Clark-Collip ('25) modification of the method of Kramer and Tisdall for determining the serum calcium of blood was used. Phosphorus was obtained by using a 1 cc. aliquot of the same solution, after which the procedure used was the same as in the method of Fiske and Subbarow ('25) for determining the inorganic phosphorus in blood serum after the blood proteins had been precipitated. Fat was determined by the Babcock test, except when the sample was small, in which case the ether extract method was employed. Protein was found by obtaining the nitrogen by the Kjeldahl method and multiplying by the factor 6.38.

COMPOSITION OF SOW'S MILK

The composition of colostrum and normal milk was studied. Colostrum was considered to be that produced during the first 60 hours after parturition and normal milk that produced from 96 hours after farrowing until the end of lactation. Between these designated times, a change takes place in the composition of the secretion that has been termed 'transition milk.' The percentage composition of these types of milk as presented by other workers is presented in tables 2 and 3. The percentage of total solids and protein of colostrum in table 2 are much higher than in normal sow's milk in table 3;

TABLE 2

Composition of colostrum

NUMBER OF SOWS	TOTAL SOLIDS	FAT	PROTEIN[1]	MILK SUGAR	ASH	REPORTED BY
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	
1	29.87	9.53	15.56	3.84	0.85	von Gohren (1865)
2	24.66	4.04	15.86	1.77	0.71	Ostertag and Zuntz ('08)
19	....	6.43	....	....	...	Hempel and Ohligmacher[2]
11	....	...	16.27	...	...	Ohligmacher[2]
Average	*26.40*	*6.35*	*16.16*	*2.46*	*0.76*	

[1] In arriving at the amount of protein von Gohren and Hempel and Ohligmacher used the factor 6.25, while Ostertag and Zuntz used 6.37.

[2] Reported by Schmidt and Lauprecht ('26).

and while the fat content varied within wide limits, the average was 6.35 per cent. Ostertag and Zuntz ('08) found the albumin and globulin content of colostrum was exceedingly high, being nearly twice that of the casein. They reported casein as 4.69 per cent and the albumin and globulin taken together as 9.09 per cent. Hempel and Ohligmacher[1] obtained a fat content of 7.38 per cent from ten sows with their first and second litters, while nine sows in their third and sixth lactations showed a content of 5.38 per cent. Hempel reported the fat content of colostrum taken from any one nipple varied little in an individual sow. Ohligmacher found the protein

[1] Reported by Schmidt and Lauprecht ('26).

content of colostrum was 16.27 per cent; that the percentage of protein falls rapidly—at the end of 2 days after parturition the average of three sows was 7.25 per cent. The same worker found four sows during a 10 weeks' lactation period fed a high-protein diet produced milk that contained 7.83 per cent

TABLE 3

Composition of normal milk

NUMBER OF SOWS	NUMBER OF SAMPLES	TOTAL SOLIDS	FAT	PROTEIN[1]	MILK SUGAR	ASH	REPORTED BY
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	
2	2	13.87	1.48	7.91	2.65	1.14	Scheven[2]
1	2	15.15	2.98	9.29	2.19	0.79	von Gohren (1865)
1	1	17.07	6.88	6.89	2.01	1.29	Lintner[2]
2	3	18.23	5.59	5.89	5.58	0.87	Cameron[2]
1	1	17.54	9.23	5.09	1.68	1.53	Ivon[2]
12	17	....	7.56	...	...	...	Petersen and Oetken (1896 a)
8	9	....	5.03	...	...	...	Petersen and Oetken (1896 b)
..	22	....	6.59	...	...	...	Petersen and Oetken (1897)
4	7	19.08	7.06	6.20	4.75	1.07	Henry and Woll (1897)
5	5	17.72	5.97	5.12	5.81	0.82	Woll (1899)
1	1	14.58	4.55	5.60	3.34	1.09	Ramoot and Braun[2]
12	24	19.49	6.89	6.06	5.64	0.98	Carlyle ('03)
1	1	16.74	5.41	4.76	5.49	1.08	Davies ('04)
2	6[3]	15.84	5.01	5.54	4.59	0.63	Ostertag and Zuntz ('08)
1	1	....	3.68	7.93	4.00	...	Folin, Dennis and Minot ('19)
..	354	....	6.99	...	...	...	Hempel and Ohligmacher[4]
..	66	....	...	6.25	...	...	Ohligmacher[4]
Aver.		*18.18*	*6.85*	*6.19*	*5.00*	*0.98*	

[1] In arriving at the amount of protein, Hempel and Ohligmacher and Ohligmacher used the factor 6.25, Ostertag and Zuntz 6.37.

[2] Analyses by Ostertag and Zuntz based on six samples for total solids and fat; five for protein; and one for milk sugar and ash.

[3] Reported by König ('03).

[4] Reported by Schmidt and Lauprecht ('26).

fat and 7.56 per cent protein, whereas seven sows fed a low protein diet gave milk with 6.63 per cent fat and 5.15 per cent protein. Normal sow's milk was found to contain (by these workers) 18.18 per cent total solids, 6.85 per cent fat, 6.19 per cent protein, 5.00 per cent milk sugar and 0.98 per cent fat. The average specific gravity of thirty-seven samples found reported was 1.0402.

In table 4 there is presented the analysis of colostrum and normal sow's milk as obtained in 1933 and 1934 at this station. The figures of the various components of colostrum vary somewhat from those in table 2; however, these differences, except for the percentage of fat, might be explained on the variation in time of taking the samples. Of the total number of samples of fat reported in table 2, nineteen were by Ohlig-macher. The sows he was working with no doubt were higher fat producers than those in this station's herd.

TABLE 4

Percentage composition of colostrum and normal sow's milk as determined at the California Experiment Station

TIME OF TAKING SAMPLE	NUMBER OF SOWS	TOTAL SOLIDS	FAT	PROTEIN[2]	ASH
Colostrum					
During parturition	13	31.86 (13)[1]	5.14 (7)	16.64 (7)	0.61 (13)
10 to 40 hours after parturition	7	23.50 (10)	5.47 (6)	12.58 (6)	0.69 (10)
Average		*28.23*	*5.29*	*14.77*	*0.64*
Normal milk					
4 to 7 days after parturition	13	18.39 (18)	5.50 (9)	5.57 (9)	0.77 (18)
15 to 45 days after parturition	8	16.58 (20)	4.90 (10)	5.95 (10)	0.99 (20)
50 to 80 days after parturition	7	18.76 (12)	5.61 (6)	8.13 (6)	1.20 (12)
Average		*17.76*	*5.29*	*6.34*	*0.96*

[1] The numbers in parenthesis indicate the number of samples available to arrive at the average figure.

[2] The factor 6.38 was used in determining the percentage of protein.

Attention is called to the rapid change in the colostrum milk, particularly in the percentage of total solids and protein. Samples drawn during parturition contained 31.86 per cent total solids and 16.64 per cent of protein, while an average of milk taken from 10 to 40 hours later contained 23.50 per cent total solids and 12.58 per cent protein. There was a slight increase in the per cent of ash after parturition. Colostrum was found to be higher in total solids and protein, lower in ash, and slightly lower in fat than normal milk.

The percentage composition of normal milk as obtained at this station, given in table 4, is similar to the average of those reported by other workers in table 3, except that the fat content is materially lower. The individual differences of the sows under test probably account for this.

TABLE 5

Composition of the ash of colostrum and normal milk of the sow as determined at the California Experiment Station

TIME OF TAKING SAMPLE	NUMBER OF SOWS	NUMBER OF SAMPLES	ASH IN PER CENT	ASH AS PER CENT OF TOTAL SOLIDS	CALCIUM AS PER CENT OF ASH	PHOSPHORUS AS PER CENT OF ASH	CALCIUM AS PER CENT OF MILK	PHOSPHORUS AS PER CENT OF MILK	CALCIUM-PHOSPHORUS RATIO
Colostrum									
During parturition	13	13	0.61	1.97	9.17	12.99	0.055	0.078	1:1.42
10 to 40 hours after parturition	7	10	0.69	2.93	15.33	13.14	0.104	0.089	1:0.86
Average			*0.64*	*2.39*	*11.85*	*13.06*	*0.076*	*0.083*	*1:1.13*
Normal milk									
4 to 7 days after parturition	13	18	0.77	4.22	24.79	15.81	0.194	0.122	1:0.64
15 to 45 days after parturition	8	20	0.99	6.02	25.96	15.36	0.257	0.152	1:0.59
50 to 80 days after parturition	7	12	1.20	6.37	27.67	16.22	0.331	0.194	1:0.59
Average			*0.96*	*5.46*	*25.95*	*15.63*	*0.252*	*0.151*	*1:0.61*

COMPOSITION OF THE ASH

The total ash and the calcium and phosphorus content of twenty-three samples of colostrum and of fifty samples of normal sow's milk were determined. The average figures for these determinations as made at this station are reported in table 5. An examination of this table reveals certain definite differences in the total ash as well as differences in total amounts of calcium and phosphorus between colostrum and normal milk. There was a significant change in the calcium and phosphorus ratio and in the relation of ash to total solids between the milk given early and that produced later in the lactation period.

The total ash and the ash figured as per cent of total solids, increase steadily during lactation. The content of calcium in colostrum taken during parturition is only about one-third that of normal milk. It increases rapidly during the first few days of lactation. While there is less phosphorus in colostrum than in normal milk, the difference is only a slight one as compared to the difference in calcium concentration. Milk taken during parturition actually contained more phosphorus than calcium. The average of eight samples, taken 10 to 40 hours later, shows a rapid increase in calcium; however, two of the eight samples taken about 20 hours after parturition still showed a higher phosphorus than calcium content. The calcium and phosphorus content of the milk and similarly of the ash showed a slight increase late in lactation. The calcium-phosphorus ratio as found in these studies was 1:1.18 for colostrum and 1:0.61 for normal milk.

DISCUSSION

A comparison of the average composition of the milk of the sow with those reported by the associates of Rogers ('28) for the milk of various mammals shows sow's milk to be higher in total solids than that of the human, cow, and horse, similar to that of the goat, and lower than that of sheep. In protein content it exceeds that of any of the species reported; the fat content is comparable to that of the sheep and the goat, but is in excess of the milk of the human, cow, and horse. The lactose or milk sugar content is similar in these species. The ash content is about the same as that of the sheep, slightly greater than that of the goat, about one-third greater than that of the cow, and about three times greater than that of human and mare's milk. In composition the normal milk of the sow is more comparable to that of the ewe than of the other mammals. The young of both species double their birth weight in a relatively short time which may in part be a reason for this similarity. Changes occurring from colostrum to normal milk are similar in the human, the horse (Linton, '31), the cow and the sow. Soon after parturition there is a

distinct and decided decrease in the total solids and protein of the species mentioned. In the cow, mare and sow the change is rapid. As lactation progresses there seems to be a slight increase in percentage of fat of the milk of the mare and sow, with a decrease in cow's milk. In the material reported, with the exception of the milk of the sow, there is a slight and steady decrease in the ash content. The ash of sow's milk is low in colostrum and increases slightly from colostrum to normal milk and continues to increase at a slow rate throughout the lactation period.

Richmond ('20) reports that the ash of cow's milk contains 14.48 per cent calcium and 12.81 per cent phosphorus, that calcium in the milk varies from 0.094 to 0.150 per cent and phosphorus from 0.084 to 0.128 per cent. Babcock and Leach ('20) have shown the calcium and phosphorus content of the ash of the milk of the cow to be, respectively, 14.30 per cent and 10.61 per cent. Widdows et al. ('30), working with human milk, reported that an average of 154 samples contained 0.035 gm. of calcium in 100 cc. of milk and seventy-three samples contained 0.018 gm. of phosphorus in the same quantity. They show an increase in the calcium and phosphorus from the first week to the second and fourth months and then a decrease, particularly in the calcium. They found an ash content in 100 cc. of milk of 0.289 gm. during the first 2 weeks, 0.243 gm. from the second to the fourth month and 0.181 gm. from the eighth to the twelfth month, a gradual decrease during the early and a rapid decrease in the latter part of lactation.

While the total solids and protein of sow's milk fall rapidly immediately after parturition, the percentage of ash begins to increase and continues to do so through lactation. During parturition the calcium content of the ash of colostrum, when it is compared with that of normal milk, is exceedingly low and it immediately increases in percentage with the decrease of total solids and protein. The percentage of phosphorus in the ash is lower in colostrum than in normal milk, but the difference is slight compared to that of calcium. The ratio

of calcium to phosphorus as determined in these studies changes rapidly from a phosphorus content in excess of that of calcium during parturition to a ratio of about 1 : 0.60 about a week later, the ratio remaining constant thereafter.

SUMMARY

Sows produce about 6.8 pounds of milk per day for an average lactation period of 8 to 12 weeks.

The averages for the constituents of colostrum as determined in these studies were: total solids 28.02 per cent, fat 5.96 per cent, protein 15.49 per cent, and ash 0.65 per cent.

The averages for normal milk as obtained were: total solids 17.98 per cent, fat 6.77 per cent, protein 6.22 per cent, and ash 0.97 per cent.

The average calcium and phosphorus content of colostrum of sow's milk as found at this station were 0.076 per cent and 0.083 per cent, respectively; while for normal milk they were: calcium 0.252 per cent and phosphorus 0.151 per cent.

Like cow's milk, the milk of the sow, at parturition, contains large quantities of total solids and protein, but both fall rapidly immediately thereafter.

The ash content of sow's milk is relatively low at parturition and increases during lactation.

The calcium and phosphorus content of normal sow's milk is higher than that reported for the human or the cow.

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MINERAL EXCHANGES OF MAN

V. BALANCES OF ELECTROLYTES IN A CASE OF HYPERPARATHYROIDISM

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FIVE FIGURES

(Received for publication August 9, 1934)

The disordered metabolism of calcium and phosphorus in hyperparathyroidism has been studied intensively since Mandl ('26) found that a case of generalized osteitis fibrosa (Recklinghausen) was improved by removal of a parathyroid tumor. The literature dealing with metabolic and other phases of the subject has been reviewed by Barr and Bulger ('30) Hunter ('31) and by Jaffe ('33).

Criteria upon which a presumptive diagnosis of parathyroid tumor may be made are now well recognized. Briefly stated, these are: hypercalcemia, hypophosphatemia, hypercalcinuria and x-ray evidence of generalized bone disease which in the milder or earlier cases may be nothing more than thinning of the bones and osteoporosis, while in the more advanced stages multiple bone cysts or giant cell tumors occur.

The opportunity to study some aspects of the metabolism of inorganic salts in a patient with osteitis fibrosa, from whom a parathyroid tumor was subsequently removed at operation prompted the present report. The details of the clinical history have been given in a separate communication (Morton, '33) and need not be repeated here, except to state that the diagnostic criteria mentioned in the preceding paragraph were fulfilled.

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METHODS

Balance studies were conducted in the metabolism unit of the hospital under carefully controlled conditions. During the greater part of the experiment the diet received by the patient was sampled and analyzed in order to determine intake. The methods employed in analysis of food and excreta have been reported in detail previously (Bassett, Elden and McCann, '31; Bassett and Van Alstine, '35). Calcium of the serum was determined by Clark and Collip's ('25) modification of the Kramer and Tisdall procedure; inorganic phosphorus was determined by the method of Kuttner and Cohn ('27). Metabolic periods were 6 days in length. Balances of nitrogen, calcium, magnesium, sodium, potassium, phosphorus and chlorine were computed for each period.

Two types of diet were used. The first contained an abundant supply of calcium and phosphorus; the second was deficient in calcium, but contained a moderate amount of phosphorus. By causing the patient to ingest each of these diets in turn for a sufficient length of time, it was hoped that the severity of the metabolic disturbance could be evaluated and that the information thus obtained would serve as a guide for subsequent therapy. Average daily intakes of protein, carbohydrate, fat, calcium and phosphorus; the Ca : P ratio; excess of acid or base in the ash of the food and calories for each diet are recorded in table 1. In order to avoid any marked gain or loss in weight, the caloric intake was adjusted to the energy expenditure of the patient in such a manner that it provided for her basal requirement and allowed a sufficient surplus to permit a moderate amount of bodily activity. Weight fluctuated within a comparatively narrow range (48.5 to 51.6 kg.). Her general health was excellent at all times.

METABOLISM OF CALCIUM AND PHOSPHORUS

Balances of calcium and phosphorus during periods in which the high and low calcium diets were ingested are shown in figure 1. Each element has been expressed in equivalents of

N/10 solution, in order that the rather close relationship between retention of calcium and phosphorus in the body or their loss from it may be more clearly appreciated. In constructing figure 1, the phosphorus balance has in each instance been corrected for retention or loss of phosphorus with nitrogen after the method described by Aub et al. ('29). The partition of calcium and phosphorus between urine and feces is shown in the data assembled in table 2. During control periods

TABLE 1

Average daily intake of food, calcium and phosphorus, including Ca: P ratios and the estimated excess of acid or base in the ash of the food

DIET	PROTEIN	CARBOHYDRATE	FAT	CALORIES	CALCIUM	PHOSPHORUS	RATIO Ca : P	EXCESS ACID[1] OR BASE ML. N/10	REMARKS
	gm.	gm.	gm.		gm.	gm.			
High calcium	78	178	109	2059	1.42	1.80	0.79	Base + 214	Control periods 11, 12, 13, 17 and 18
	78	178	109	2059	1.42	3.87	0.36	Acid + 319	Sodium acid phosphate periods 14, 15 and 16
Low calcium	50	179	137	2226	0.148	0.706	0.21	Acid + 164	Controls periods 19, 20 21, 25 and 26
	50	179	137	2226	1.48	2.80	0.53	Acid + 38	Calcium salts and acid phosphate—periods 22, 23 and 24

[1] The acidifying effect of ingestion of sodium acid phosphate and the alkalinizing effect of calcium lactate and gluconate have been included in estimating the excess of acid or base in the food. The high Ca and P intake shown in periods 22, 23 and 24 was due to superposition of calcium lactate, calcium gluconate and sodium acid phosphate on the low calcium diet.

the retentions of both elements diminished in successive periods, although the intake of each remained essentially constant.

Effect of ingestion of $NaH_2PO_4.H_2O$ on balance. The addition of sodium acid phosphate to the diet in the amounts indicated (fig. 1 and table 2) caused a considerable increase in the retention of both elements. The immediate effect was to produce a large increase in the retention of phosphorus in excess of the amount which could have combined with

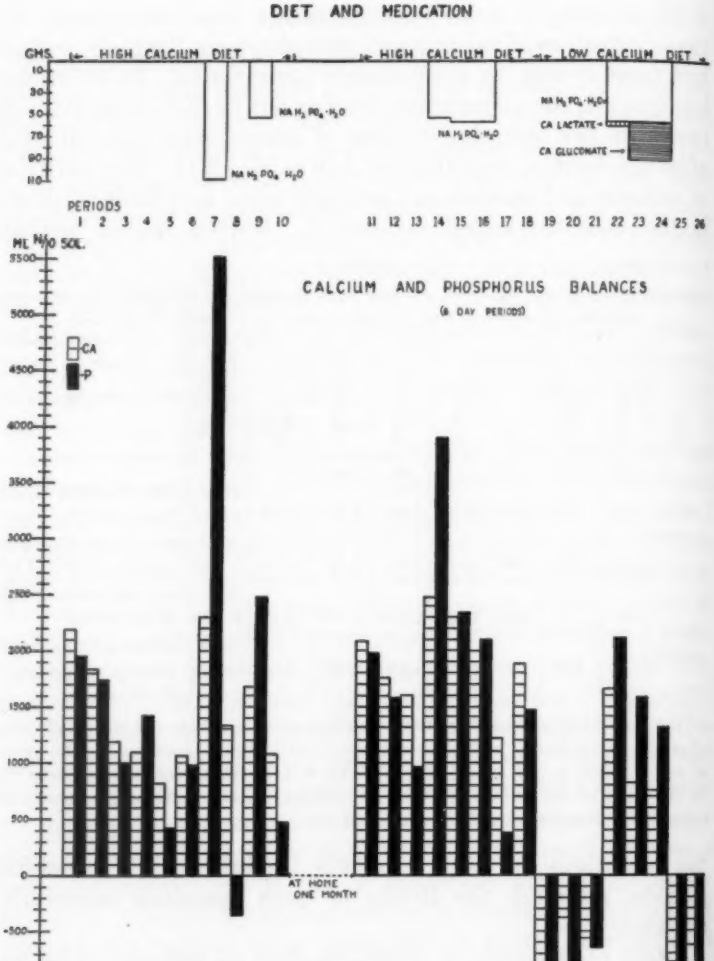
DIET AND MEDICATION
GMS.
HIGH CALCIUM DIET
HIGH CALCIUM DIET
LOW CALCIUM DIET
NA H2 PO4 · H2O
NA H2 PO4 H2O
NA H2 PO4 · H2O
CA LACTATE
CA GLUCONATE
PERIODS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
ML N/10 SOL.
CALCIUM AND PHOSPHORUS BALANCES
(6 DAY PERIODS)
CA
P
AT HOME ONE MONTH

Fig. 1 Only the amounts of calcium or phosphorus retained in or lost from the body are shown in the graph. Phosphorus has been considered trivalent and each balance has been corrected for phosphorus presumably retained or lost with nitrogen (Aub et al., '29).

calcium to form $Ca_3(PO_4)_2$ and to decrease the excretion of calcium in the urine. When the administration of acid phosphate was continued in consecutive periods (14 to 16) the excretion of phosphorus in the urine gradually increased and the retentions of phosphate decreased. In the first control period which followed (period 17) comparatively little phosphorus was retained and the retention of calcium also decreased, but not to a similar extent. The inequalities in the retention of calcium and phosphorus in individual periods show that either one may be retained for a time in excess of the other. However, when the total retentions of each element were compared over a longer interval of time (periods 11 to 18, inclusive) it was found that 30.78 gm. of calcium and 15.27 gm. of phosphorus (excluding phosphorus deposited with nitrogen) were retained. The Ca : P ratio was $\frac{30.79}{15.27} = 2.01$ which is almost identical with the Ca : P ratios found in bone.

Effect of sodium acid phosphate on concentrations of calcium and inorganic phosphorus in the serum. Hypercalcemia was at no time extreme; on the other hand, very low values of inorganic phosphorus in serum were frequently observed (table 3). As Bulger, Dixon, Barr and Schregardus ('30) and Albright, Bauer, Claflin and Cockrill ('32) have shown, the high values of calcium could be reduced toward normal and the low values of inorganic phosphorus were increased by the administration of acid phosphate. These effects, as Albright and collaborators noted, were dependent upon the amount of acid phosphate administered. Large doses of phosphate (period 7) caused the inorganic phosphorus of the serum to rise and the level of calcium in serum to fall nearly to normal. When approximately half of this large dose was employed (period 9) the effect on the levels of Ca and P in the serum was less pronounced. In both instances there was increased retention of calcium and phosphorus in the body; the larger dose produced the more marked retention. In general, then, the effects of administration of acid phosphate were similar to those noted by others who have

TABLE 2

Exchanges of nitrogen, calcium, phosphorus and magnesium

PERIODS (6 DAYS)	NITROGEN			CALCIUM			PHOSPHORUS			MAGNESIUM			COMMENT
	Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	
		U. 64.35			U. 3.33			U. 6.90			U. 0.889		
		F. 5.05			F. 0.98			F. 1.25			F. 0.635		Control period
11	75.33	T. 69.40	+ 5.93	8.50	T. 4.31	+ 4.19	10.50	T. 8.15	+ 2.35	1.723	T. 1.524	+ 0.199	High calcium diet
		U. 68.47			U. 3.64			U. 7.21			U. 0.863		
		F. 3.52			F. 1.27			F. 1.28			F. 0.674		Control period
12	75.41	T. 71.99	+ 3.42	8.43	T. 4.91	+ 3.52	10.30	T. 8.49	+ 1.81	1.668	T. 1.537	+ 0.131	High calcium diet
		U. 66.90			U. 3.41			U. 7.16			U. 0.828		
		F. 4.63			F. 1.57			F. 1.70			F. 0.691		Control period
13	75.09	T. 71.53	+ 3.56	8.19	T. 4.98	+ 3.21	10.05	T. 8.86	+ 1.19	1.668	T. 1.519	+ 0.149	High calcium diet
		U. 68.87			U. 2.17			U. 17.24			U. 0.691		
		F. 3.90			F. 0.98			F. 1.39			F. 0.648		As above
14	76.94	T. 72.77	+ 4.17	8.11	T. 3.15	+ 4.96	22.87	T. 18.63	+ 4.24	1.676	T. 1.339	+ 0.337	+ 52 gm. $NaH_2PO_4.H_2O$
		U. 69.35			U. 2.15			U. 18.72			U. 0.712		
		F. 4.92			F. 1.60			F. 2.15			F. 0.943		As above
15	77.06	T. 74.27	+ 2.79	8.36	T. 3.75	+4.61	23.43	T. 20.87	+ 2.56	1.668	T. 1.655	+ 0.013	+ 56 gm. $NaH_2PO_4.H_2O$
		U. 68.95			U. 2.42			U. 18.90			U. 0.706		
		F. 3.45			F. 1.83			F. 2.03			F. 0.844		As above
16	79.25	T. 72.40	+ 6.85	8.33	T. 4.25	+ 4.08	23.44	T. 20.93	+ 2.51	1.701	T. 1.550	+ 0.151	+ 56 gm. $NaH_2PO_4.H_2O$
		U. 66.80			U. 4.42			U. 8.56			U. 0.911		
		F. 3.86			F. 1.75			F. 1.75			F. 0.790		Control period
17	79.44	T. 70.66	+ 8.78	8.60	T. 6.17	+ 2.43	11.16	T. 10.31	+ 0.85	1.643	T. 1.701	— 0.058	High calcium diet

		U. 69.09 F. 3.07			U. 4.50 F. 1.34			U. 7.91 F. 1.22			U. 0.872 F. 0.541		Control period High calcium diet
18	78.75	T. 72.16	+ 6.59	9.63	T. 5.84	+ 3.79	10.99	T. 9.13	+ 1.86	1.748	T. 1.413	+ 0.335	
		U. 61.41 F. 2.26			U.1.62 F. 0.85			U. 4.88 F. 1.00			U. 0.546 F. 0.640		Control period Low calcium diet
19	55.39	T. 63.67	— 8.28	0.75	T. 2.47	— 1.72	4.32	T. 5.88	— 1.56	1.124	T. 1.186	— 0.062	
		U. 51.63 F. 3.87			U. 1.65 F. 0.12			U. 4.43 F. 0.75			U. 0.476 F. 0.703		Control period Low calcium diet
20	57.81	T. 55.50	+ 2.31	1.02	T. 1.77	— 0.75	4.42	T. 5.18	— 0.76	1.204	T. 1.179	+ 0.025	
		U. 50.66 F. 4.13			U. 1.80 F. 0.21			U. 4.13 F. 0.70			U. 0.479 F. 0.676		Control period Low calcium diet
21	56.48	T. 54.79	+ 1.69	0.90	T. 2.01	— 1.11	4.25	T. 4.83	— 0.58	1.150	T. 1.155	— 0.005	
		U. 47.04 F. 3.46			U. 1.35 F. 4.50			U. 12.07 F. 2.14			U. 0.341 F. 0.696		As above + 60 gm. calcium lactate
22	56.13	T. 50.50	+ 5.63	9.18	T. 5.85	+ 3.33	16.68	T. 14.21	+ 2.47	1.152	T. 1.037	+ 0.115	+ 56 gm. $NaH_2PO_4.H_2O$
		U. 52.65 F. 3.16			U. 1.30 F. 5.81			U. 13.25 F. 2.05			U. 0.403 F. 0.720		As above + 90 gm. calcium gluconate
23	54.73	T. 55.81	— 1.08	8.77	T. 7.11	+ 1.66	16.90	T. 15.30	+ 1.60	1.208	T. 1.123	+ 0.085	+ 56 gm. $NaH_2PO_4.H_2O$
		U. 52.90 F. 3.28			U. 1.22 F. 6.00			U. 13.26 F. 2.26			U. 0.384 F. 0.641		As above + 90 gm. calcium gluconate
24	54.49	T. 56.18	— 1.69	8.74	T. 7.22	+ 1.52	16.82	T. 15.52	+ 1.30	1.172	T. 1.025	+ 0.147	+ 56 gm. $NaH_2PO_4.H_2O$
		U. 52.36 F. 2.95			U. 1.64 F. 0.84			U. 4.87 F. 0.73			U. 0.526 F. 0.577		Control period Low calcium diet
25	54.20	T. 55.31	— 1.11	0.92	T. 2.48	— 1.56	4.20	T. 5.60	— 1.40	1.188	T. 1.103	+ 0.085	
		U. 49.04 F. 3.06			U. 1.70 F. 0.31			U. 4.17 F. 0.81			U. 0.536 F. 0.739		Control period Low calcium diet
26	55.29	T. 52.10	+ 3.19	0.79	T. 2.01	— 1.22	4.14	T. 4.98	— 0.84	1.172	T. 1.275	— 0.103	

Legend: U = urine, F = feces, T = total. Signs: + retention in body; — loss from body.

used salts of orthophosphoric acid in this condition. The only unusual feature of this phase of the experiment appears to have been the large amounts of calcium and phosphorus which were retained when these elements were furnished in rather high concentrations in the diet. Inspection of table 2 shows that from one-third to one-half of the calcium ingested during periods 11 to 18 was deposited in the body.

Effect of a low calcium diet. The effect of decreasing the calcium of the food from 1.42 gm. to 0.15 gm. per diem re-

TABLE 3

PERIOD 6 DAYS	Ca IN SERUM MG./ PER CENT	INORGANIC P IN SERUM MG./ PER CENT	TOTAL SERUM PROTEIN GM./ PER CENT	Ca × P PRODUCT	CALCIUM BALANCE, GM.	COMMENT
2	13.30 (6)	2.38 (6)	7.66 (6)	31.6 (6)	+ 3.66	High calcium-phosphorus diet
4	13.66 (6)	1.57 (6)	6.51 (6)	21.4 (6)	+ 2.21	High calcium-phosphorus diet
7	11.35 (2) 11.17 (4)	3.59 (2) 3.02 (4)	6.75 (2) 6.73 (4)	41 (2) 33.7 (4)	+ 4.60	As above + 108 gm. $NaH_2PO_4.H_2O$
8	14.00 (3)	1.65 (3)	7.22 (3)	23 (3)	+ 2.66	Phosphate omitted
9	14.29 (1) 12.00 (4) 12.18 (6)	1.54 (1) 2.40 (4) 1.96 (6)	7.84 (1) 7.64 (4) 8.14 (6)	22 (1) 28.8 (4) 23.9 (6)	+ 3.36	As above + 52.8 gm. $NaH_2PO_4.H_2O$
10	12.44 (2)	1.63 (2)	6.67 (2)	20.3 (2)	+ 2.16	Phosphate omitted

Numbers in parenthesis show day of period on which sample of blood was taken. Values for first day of period were obtained prior to administration of sodium acid phosphate in that period.

+ = Calcium retained.

sulted as had been expected in a loss of calcium, presumably from the bones. The phosphorus balance ran essentially parallel with the calcium balance, although there was no absolute deficit of phosphorus in the food (table 1). A more surprising feature of this phase of the experiment was the fact that more phosphorus was lost than the loss of calcium seemed to warrant, assuming, of course, that these elements were being withdrawn from the bones. This excess loss of phosphorus was not due to a negative nitrogen balance, since

the patient remained essentially in nitrogen equilibrium. Calcium and phosphorus in the form of calcium lactate and calcium gluconate and acid sodium phosphate were now added to the ration in amounts sufficient to provide a level of intake comparable to that which had been given when the high calcium-phosphorus diet was ingested. Calcium was given at first as the lactate, but was discontinued and equivalent amounts of gluconate were substituted when it was found that calcium lactate produced nausea. Care was taken not to give the calcium and phosphorus salts together. The calcium was administered in divided doses and given with the meals. Phosphate was given four times daily and at least 2 hours before or after a meal. At this higher level of intake both elements were retained. Again the balances paralleled each other, but more phosphorus was retained than could have been utilized in the formation of $Ca_3(PO_4)_2$. With the omission of salts of calcium and phosphorus in the final control periods, the findings noted in the initial control periods were duplicated. Increasing the amounts of calcium and phosphorus in the ration (periods 22, 23 and 24) produced little change in the concentrations of Ca and inorganic P in the serum, yet the differences in urinary and fecal excretion of the two elements were of interest. The excretion of calcium in the urine was at the lowest level observed and the excretion in the feces which had previously been at an extraordinarily low level was markedly increased. The increased excretion of fecal calcium was probably due for the most part to calcium which had escaped absorption. While it is a temptation to speculate concerning possible reasons for the relatively poorer absorption of these salts (calcium lactate and gluconate) than of the calcium of the food, the data at hand are not sufficiently complete to permit conclusions to be drawn.

The partition of phosphorus between urine and feces remained essentially unaltered. The ratio, phosphorus in urine: phosphorus in feces was 5.5:1 in control periods and 5.9:1 at the higher level of intake of calcium and phosphorus. Throughout the entire experiment the excretion of phosphorus

in the feces seems to have shown a tendency to parallel the amount of phosphorus in the ration and to have been fairly independent of the excretion of calcium in the feces. The latter observation is in agreement with the opinion expressed by Bulger and collaborators ('30, '31).

Magnesium. The nature of the bone lesions in hyperparathyroidism is such that one would expect to find the concentration of magnesium salts in bone reduced as are those of calcium. In a patient studied by Wilder ('29) the amount of magnesium in a specimen of bone removed at biopsy was normal, while the organic matter was increased and its content of calcium and phosphorus was definitely decreased. Bulger and Gausmann ('33), in a balance experiment, demonstrated a tendency for an individual with hyperparathyroidism to lose magnesium from the body. When a parathyroid tumor was removed, large amounts of calcium, phosphorus and magnesium were retained. No consistent changes in the concentration of magnesium in the blood were observed.

Data on the exchanges of magnesium in our patient are shown in figure 2 and table 2. Attention is called to the fact that, 1) approximate equilibrium between intake and outgo of magnesium was attained in periods 1 to 10 during which considerable amounts of calcium and phosphorus were stored; 2) that at a slightly higher level of magnesium intake (periods 11 to 19), there was retention of magnesium and, 3) that when the shift was made to the diet deficient in calcium (periods 19, 20 and 21), although there was coincidently a reduction in the intake of magnesium, no loss of magnesium from the body occurred. The exchanges of magnesium, therefore, showed considerable independence of the calcium in the food and of the calcium balance.

The administration of sodium acid phosphate affected the partition of magnesium between urine and feces. Large doses of phosphate produced a decrease in the excretion of magnesium in the urine (periods 7, 9, 14, 15 and 16) which was partly offset in some periods (9, 15 and 16) by an increased excretion of magnesium in the feces. The large fecal excre-

tion of base (particularly calcium) in periods 22, 23 and 24 seems to have prevented an increase in the excretion of fecal magnesium (fig. 3). The quite definite decrease in urinary magnesium in these periods (presumably produced by phosphate) resulted in retention of magnesium. While these observations do not point to any characteristic abnormality in

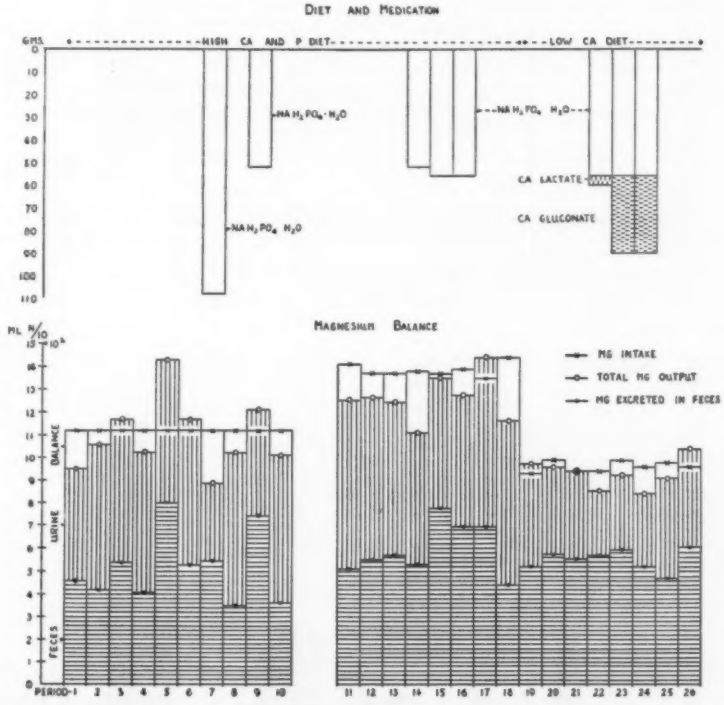

Fig. 2 Magnesium balance expressed as milliliter of N/10 solution (units of the scale $\times 10^3$).

the partition of magnesium between urine and feces in hyperparathyroidism, nor to an excessive loss of this metal from the body during the course of the disease, we have subsequently been able to show that removal of a parathyroid tumor from the patient was followed by retention of considerable amounts of magnesium.

Sodium and potassium. Approximate equilibrium between intake and outgo of sodium and potassium was maintained throughout the experiment. Small positive and negative

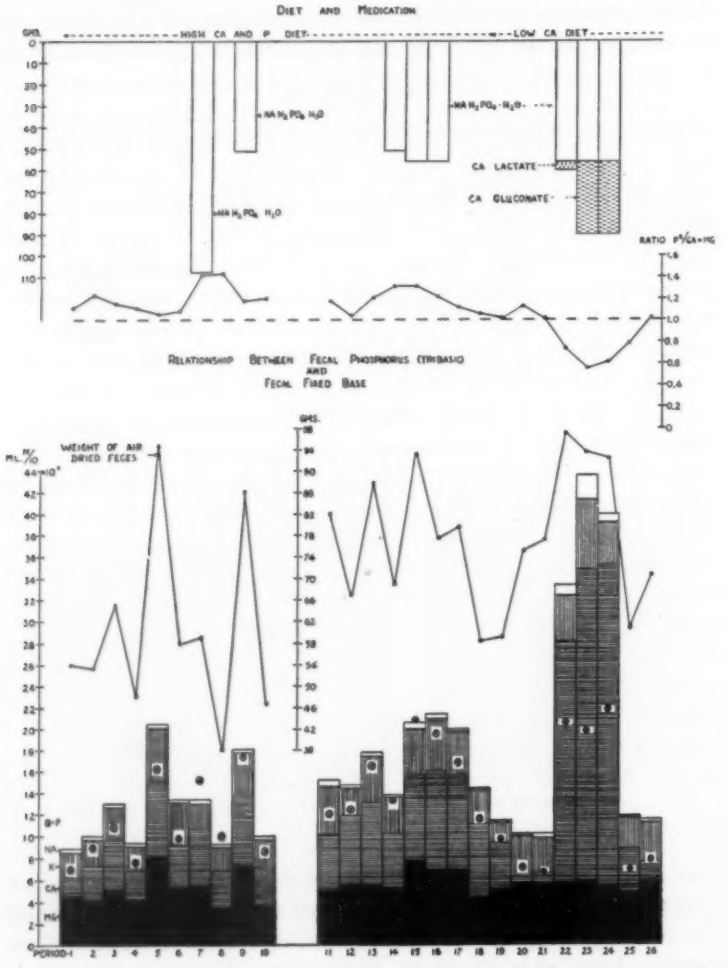

Fig. 3 Excretion of fixed base and phosphorus in the feces expressed as milliliter of N/10 solution. (Units of scale × 10^3). Phosphorus has arbitrarily been considered trivalent.

balances occurred in individual periods. Some degree of correlation was observed between balances of sodium (when calculated as extracellular water) and the fluctuations which occurred in body weight. A similar correlation between small retentions and losses of potassium and changes in the weight of the body could not be demonstrated.

TABLE 4

Effect of administration of sodium acid phosphate on sodium excretion in urine

PERIOD	SODIUM EXCRETION IN URINE ML. N/10	SODIUM EXCRETION IN EXCESS OF CONTROL ML. N/10	EXTRA SODIUM GIVEN WITH $NaH_2PO_4.H_2O$ ML. N/10	MEDICATION
Average of 12 and 13	[1] 7,560			None
14	11,890	4,330	3,869	$NaH_2PO_4.H_2O$ 52.29 gm.
15	11,780	4,220	4,056	$NaH_2PO_4.H_2O$ 55.96 gm.
16	12,525	4,965	4,043	$NaH_2PO_4.H_2O$ 55.82 gm.
17	7,510	— 50		None
Totals for 14, 15, 16 and 17		13,465	11,959	
Average of 21 and 26	[1] 5,376			None
22	9,008	3,632	4,039	Calcium lactate 60 gm. $NaH_2PO_4.H_2O$ 55.78 gm.
23	9,504	4,128	4,074	Calcium gluconate 90 gm. $NaH_2PO_4.H_2O$ 56.23 gm.
24	8,839	3,463	4,087	Calcium gluconate 90 gm. $NaH_2PO_4.H_2O$ 56.40 gm.
25	6,026	650		None
Totals for 22, 23, 24 and 25		11,873	12,200	

[1] Control periods: periods 12 to 17, inclusive, high calcium diet; periods 21 to 25, inclusive, low calcium diet.

The excretion of potassium was not affected by the ingestion of as much as 18 gm. of sodium acid phosphate per diem for 6 days.

Extra sodium administered as the acid phosphate was almost quantitatively excreted in the urine (table 4) either during the period in which it was ingested or during the first after period.

Fecal sodium was not materially increased during periods of ingestion of phosphate (fig. 3), although, as previously stated, the absolute amount of fecal phosphorus was considerably increased. We have found that a normal subject, while ingesting a similar diet, failed to show an increased excretion of sodium in the feces even when the amount of fecal phosphorus was more than doubled by administration of sodium acid phosphate. This seems to disprove the contention of Salter, Farquharson and Tibbetts ('32) that the administration of sodium orthophosphate leads to the excretion of considerable amounts of disodium phosphate in the feces.

Balance of acid and base in urine. The data on excretion of acid and base in the urine are represented graphically in figure 4. Since organic acids and bicarbonate were not actually determined, it has been assumed that they were equal to the difference between the sum of the basic components and the total excretion of inorganic acids. No distinctive features were noted in control periods other than the unusually large excretion of calcium and phosphorus. The changes in urinary acid and base produced by the administration of $NaH_2PO_4.H_2O$ were similar to, if not identical with, those described by Farquharson, Salter and Aub ('31) in urine of normal subjects who ingested acid phosphate. In brief, it may be stated that in uncomplicated hyperparathyroidism the excretion of acid and base in the urine conforms closely to the normal pattern. This substantiates the work of Albright, Bauer and Aub ('31).

DISCUSSION

Sodium and potassium. Greenwald ('29) has suggested that the retention of base (sodium and potassium) which follows parathyroidectomy may be due to a disturbance of renal function brought about by the lowered concentration of calcium in the serum. Experiments of Albright and Ellsworth ('29) and Ellsworth ('32) strongly suggest that parathormone has an effect on renal function, since a diuresis of phosphorus and lowering of the concentration of inorganic phosphorus in the serum promptly follow injection of the hormone. These ef-

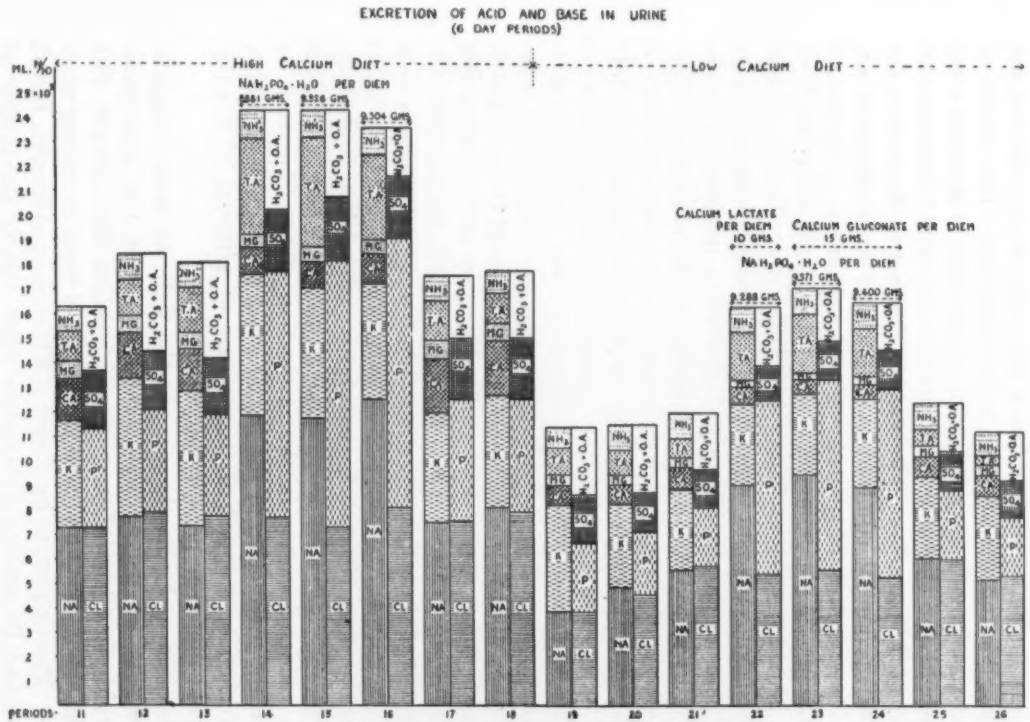

Fig. 4 Acid-base balance in urine expressed as milliliter of N/10 solution. (Units of scale $\times 10^2$.) The organic acid (O.A.) and bicarbonate have been estimated as the difference between the sum of the cations, which include ammonia and titratable acid, and the sum of the anions Cl, P and SO_4.

fects were noted prior to an increase in the concentration of calcium in the serum and hence the latter is presumably not responsible for alteration of kidney function. The results of their experiments have led them to postulate a lowered renal threshold for phosphorus as perhaps the most fundamental effect of the parathyroid hormone. Further evidence that the disturbance in the equilibrium of electrolytes produced by excessive excretion of phosphorus and calcium is accompanied by changes in the balances of other inorganic salts has been furnished by Albright, Bauer and Aub ('31). They produced hyperparathyroidism in a patient with myositis ossificans by injections of parathormone and observed some increase in the excretion of water, base (in addition to calcium) and chloride. A reversal of these phenomen occurred when the injections of the hormone were discontinued. In so far as the present study of uncomplicated hyperparathyroidism is concerned, we were unable to demonstrate any tendency toward excessive excretion or retention of sodium, potassium or chlorine, nor were there any alterations in the normal partition of these elements between urine and feces. Our results are not incompatible with the observations of Albright and collaborators, since the effects that these investigators describe appear to have been of a temporary nature. Noteworthy changes in the balances of water and electrolytes (exclusive of calcium and phosphorus) are probably to be expected only at times when there is a sudden increase in the production of parathormone or when the production of the parathyroid secretion is suddenly decreased or stopped as in the case of removal of a parathyroid tumor or parathyroidectomy.

Calcium and phosphorus. In the light of more complete knowledge of the ultimate consequences of the hyperparathyroid state, the advisability of treating this disease with high calcium diets and acid phosphate is open to serious question. Recent papers by Bauer ('33) and by Albright, Baird, Cope and Bloomberg ('34) show quite clearly the futility of treatment in some cases and the complications which may arise in others when attempts are made to prevent further

decalcification of the skeleton or to cause healing of the bone lesions by ingestion of a high calcium diet. While it is admitted that suitable dietary measures can cause deposition of calcium salts in the bones of the milder cases of hyperparathyroidism, Albright and collaborators ('34) report the development of considerable impairment of renal function in such a patient who had been treated by the administration of

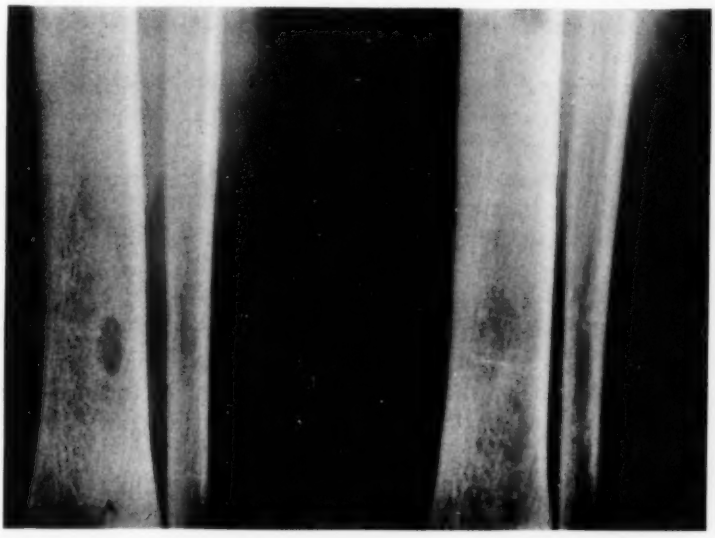

Fig. 5 A) Roentgenogram of shaft of left tibia prior to administration of a high calcium-phosphorus diet. B) Shaft of right tibia after 3 months on high calcium-phosphorus diet, showing calcification of cyst.

a high calcium diet. They show evidence which leads to the conclusion that the renal injury was produced by the formation of pathological calcifications in the tissues of the kidney or within the renal tubules. That conditions which favor the formation of these deposits may well be produced or enhanced by the ingestion of a high calcium diet is shown admirably in the case of our patient. During control periods in which she received food rich in calcium, both the quantity and con-

centration of calcium in the urine was two- to threefold greater than in similar control periods in which she ingested food poor in calcium. Fortunately, the urea clearance test (Van Slyke) (Peters and Van Slyke, '31) and creatinine filtration rate (Rehberg) (Holton and Rehberg, '31) showed no impairment of renal function after the patient had ingested a high calcium diet for nearly a year. At the conclusion of this period of treatment, one bone cyst had recalcified (fig. 5). However, a roentgenogram showing the proximal end of the shaft of the same bone disclosed the formation of a new cystic area which had apparently developed simultaneously with the healing of the cyst in the more distal portion of the shaft. Furthermore, metabolic data suggest that the immediate storage of calcium and phosphorus in the body which followed the institution of a high calcium phosphorus regimen was not well sustained. This is illustrated by the fairly rapid and apparently progressive decrease in the positive balances of these elements which took place in consecutive control periods.

The evidence cited, together with the fact that there was no indication, after more than a year of observation, of abatement in the severity of the metabolic disorder, led to a decision to explore the region of the thyroid in search of a parathyroid tumor. Metabolic studies carried on at the time the tumor was discovered and removed are reported separately.

SUMMARY

1. The ingestion of a diet rich in calcium and phosphorus by a patient with mild hyperparathyroidism resulted in retention of these elements in approximately the proportions in which they occur in bone. During 108 days the total amount of calcium retained was 60 gm., of phosphorus 34.7 gm. and of nitrogen 70 gm. In spite of this, the ingestion of a high calcium diet for 1 year did not result in appreciable recalcification of the skeletal lesions.

2. The daily administration of from 10 to 18 gm. of sodium acid phosphate increased the concentration of inorganic phosphorus in blood plasma and decreased hypercalcemia and

hypercalcinuria. The amount of calcium and phosphorus retained was increased during the periods in which acid phosphate was ingested. These effects were temporary and ceased as soon as the administration of phosphate was discontinued.

3. Calcium of the food was much more completely absorbed than calcium administered in the form of lactate or gluconate.

4. The ingestion of a low calcium diet was attended by a marked decrease in the amount of calcium excreted in the urine.

5. The exchanges of magnesium were not materially influenced by the state of the calcium balance.

6. The ingestion of large amounts of sodium acid phosphate caused a decrease in the amount of magnesium excreted in the urine and in several instances an increase in the excretion of magnesium in the feces.

7. Extra phosphoric acid, administered as sodium acid phosphate, was excreted chiefly in the urine. Provision was made for the elimination of this excess of acid by, a) increase in the titratable acid in the urine, b) decrease in the excretion of undetermined acid in the urine (bicarbonate plus organic acid) and, c) by an increase in excretion of fixed base (sodium) in amounts equivalent to those present in the sodium acid phosphate which was ingested.

8. The excretion of fecal phosphorus showed a slight increase as a result of administration of phosphate, and there was usually a concomitant increase in the excretion of fecal fixed base in the form of calcium and magnesium. There was little or no correlation between excretion of phosphorus in the feces and the fecal excretion of sodium and potassuim.

9. Sodium ingested with the food, as a solution of sodium chloride and as sodium acid phosphate was eliminated almost quantitatively in the urine.

10. Equilibrium between intake and outgo of potassium was maintained throughout the period of observation.

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MINERAL EXCHANGES OF MAN

VI. THE EFFECT OF EXTIRPATION OF A PARATHYROID TUMOR ON THE BALANCES OF ELECTROLYTES

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FOUR FIGURES

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Metabolic experiments performed by Greenwald ('13) and Greenwald and Gross ('25) provided the first satisfactory evidence of the effect of parathyroidectomy on the excretion of calcium and phosphorus. That retention of these elements follows the removal of the parathyroid glands has been confirmed repeatedly, but it is less clearly established that this operation is capable of influencing the metabolism of other inorganic constituents of the body.

In one of his earlier experiments Greenwald ('13) noted that primary retention of phosphorus was followed by a secondary decrease in the excretion of sodium and potassium in the urine. He confirmed this observation later (Greenwald, '29) and suggested by way of explanation that both "the increased concentration of inorganic phosphorus in the serum and the diminished excretion of sodium and potassium" might be due to an "interference with renal function brought about by the lowered calcium concentration in the plasma."

Although removal of a parathyroid tumor for therapeutic purposes seems to have many points in common with experimental parathyroidectomy in animals (Bulger, Dixon, Barr

[1] Aided by a grant from the Fluid Research Fund contributed by the Rockefeller Foundation.

and Schregardus, '30) even on occasion being followed by severe tetany (Bulger et al., loc. cit.; Snapper, '30 and others) the question of its effect on the metabolism of sodium and potassium does not appear to have been investigated. Evidence that the parathyroid hormone has some action on the mechanisms which regulate the volume of body water in man is not altogether lacking. A diuretic action has been attributed to parathormone by several of those who have investigated its therapeutic and physiological potentialities (Davidson, '25; Mason, '26; McCann, '28; Johnson, '31). A metabolic study by Albright, Bauer and Aub ('31) showed that when hyperparathyroidism was produced by the injection of parathormone, a small increase in the excretion of water, chloride and base followed the initial injections, with subsequent retention of these elements when the treatment was discontinued. They stated that no evidence was obtained which indicated a fundamental disturbance in the acid-base balance of the body. We have previously given attention to this phase of metabolism without being able to detect losses of sodium or potassium during the hyperparathyroid state (Bassett, '35). While such losses if small might easily have been obscured by errors in experimental technic, it seems more probable, as the data of Albright and co-workers suggest, that changes in the volume of water and its solutes may be limited to short periods of readjustment, which are initiated by sudden increases or decreases in the amount of parathormone made available to the organism. In order to subject this hypothesis to further test, we have followed the exchanges of sodium, potassium, calcium, magnesium, chlorine and phosphorus both before and during the critical period which follows removal of a parathyroid tumor.

EXPERIMENTAL PROCEDURE AND METHODS

The same patient, A. L. no. 48736, who served as the subject of the former investigation and whose clinical history has been reported in detail by Morton ('33), re-entered the metabolism unit of the hospital for the necessary pre-operative studies.

The mineral exchanges were followed for 21 days prior to extirpation of a parathyroid tumor and for 27 days subsequent to the operation. The patient remained in the metabolism unit throughout this time, except for a brief interval of approximately 3 hours which was spent in the operating room. The operation was accomplished with practically no loss of blood and with but slight manipulation of the thyroid apparatus. There was no visible sweating during operation nor in the period of recovery from the anaesthetic (avertin and nitrous oxide). Following operation, the patient vomited once. The amount of vomitus did not exceed 100 cc. and was added to the excreta for analysis. All collections of urine and feces were quantitative. For 3 days preceding operation the urine was saved in 12-hour day and night specimens and each of these was analyzed for sodium, potassium, calcium, chlorine and phosphorus. The same procedure was followed during the first 6 days of convalescence, except in one or two instances when, because of difficulty in voiding, the collection periods exceeded 12 hours. Other than this collections of excreta were made for the usual metabolic period of 3 or 6 days. Daily analyses of urine were then restricted to the determination of ammonia, titratable acidity minus carbonates (Albright and Bauer, '29), total organic acids (Van Slyke and Palmer, '20) and creatinine. Representative samples of each serving of food were saved, pooled, and analyzed to determine the intake of mineral elements in each period (Bassett and Van Alstine, '35). Table 1 gives the average daily intake of carbohydrate, protein, fat, calories, water, calcium and phosphorus for each metabolic period. The alterations in the level of intake in periods 31 and 32 were unavoidable, since the patient experienced some difficulty in swallowing during early convalescence and could not at once resume the diet of the fore periods.

Favorable experimental and clinical reports on the use of irradiated ergosterol for relief of parathyreoprevic tetany (Wade, '29; Brougher, '30; Bauer, Marble and Claflin, '32; Schultzer, '32) led to its use as a prophylactic measure. Ap-

proximately 3000 rat units of viosterol (Squibb) were given daily for 3 days preceding removal of the tumor and the dose was increased to 3750 units on the second post-operative day when mild tetany appeared. One intravenous injection of 50 units of parathormone was also given on this day. Tetany did not appear again in an active form, although there were occasional signs of latent tetany until the date of discharge from the hospital. Viosterol was continued through the eleventh post-operative day and then omitted.

TABLE 1

Average daily intake of protein, carbohydrate, fat, water, calories, calcium and phosphorus

PERIODS	PROTEIN	CHO	FAT	WATER[1]	CALORIES	CALCIUM	PHOSPHORUS	RATIO Ca/P	COMMENT
	gm.	*gm.*	*gm.*	*Liters*		*gm.*	*gm.*		
27, 28, 29, 30, 33, 34, 35	81	243	106	3.36	2314	1.127	1.543	0.73	21 days before operation and 9th to 26th day, inclusive, after operation
31[2]	62	203	91	3.26	1933	1.471	1.338	1.10	First 5 days after operation
32	63	142	83	2.9	1612	1.133	1.286	0.88	6th to 8th days, inclusive, after operation

[1] Water drunk and water of food.

[2] Day of operation not included, since no food was given on that day.

On the day of operation 1500 ml. of salt solution containing 0.7 per cent of sodium chloride, 0.03 per cent of potassium chloride and 0.025 per cent of calcium chloride were administered subcutaneously. This comprised practically the entire salt intake for the day. Water was given by mouth in small amounts after consciousness had been regained. The intake of fluid was thus brought to a total of 2350 ml. Thereafter liquid foods and water were ingested in moderate quantities (average daily values appear in table 1) until the sixth post-operative day when a semi-solid diet was again tolerated.

METABOLIC DATA

The actual balances which were computed for each period, exclusive of the acid-base balance of the urine, are shown in tables 2 and 3. For purposes of discussion, it will be convenient to point out the major changes produced by the removal of the parathyroid tumor and then proceed with a more detailed examination of metabolic effects in the case of single elements or groups of closely related elements.

After about 18 days of nearly constant intake of food and water and of carefully regulated activity, approximate equilibrium between intake and outgo of mineral elements was obtained. The parathyroid tumor was then excised. An immediate retention of phosphorus, base, chlorine and water followed this procedure. The remarkable daily retention of base in the early stages of recovery is seen in figure 1.

Calcium and phosphorus. The changes in the metabolism of calcium and phosphorus were most striking, but are now so well known that they require but little comment (Bulger et al., '30; Hunter and Turnbull, '31). Our findings conformed closely to those published in the literature. There was a precipitate fall in serum calcium. Within 24 hours after operation, it had declined from 12.5 to 9.2 mg. per cent. The post-operative excretion of phosphorus in the urine practically ceased for 36 hours. The excretion of calcium in the urine decreased somewhat less abruptly, but continued to decline gradually until it became established at about one-fifth of its pre-operative level. The excretion of urinary phosphorus, on the other hand, began to increase after the second post-operative day, but did not regain its former high level of excretion. Little change occurred in the concentration of inorganic phosphorus in the serum until about the time at which the urinary excretion of phosphorus became reestablished, when it increased from 2 to approximately 3 mg. per cent. The increase in the concentration of inorganic phosphorus in the serum and the increased excretion of this element in the urine, therefore, coincided almost perfectly.

TABLE 2

Balances of nitrogen, calcium, magnesium and phosphorus

PERIOD	NITROGEN, GM.			CALCIUM, GM.			MAGNESIUM, GM.			PHOSPHORUS, GM.			COMMENT
	Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	
27 6 days Jan. 9–14	Total 75.1	U 63.3 F. 4.9 68.2	+ 6.9	6.56	U. 3.55 F. 1.22 4.77	+ 1.79	2.148	U. 0.811 F. 0.933 1.744	+ 0.404	9.17	U. 5.90 F. 1.29 7.19	+ 1.98	Control periods
28 6 days Jan. 15–20	Total 75.0	U. 62.6 F. 4.9 67.5	+ 7.5	6.62	U. 3.28 F. 2.01 5.29	+ 1.33	2.136	U. 0.842 F. 1.236 2.078	+ 0.058	9.28	U. 6.69 F. 1.66 8.35	+ 0.93	Control periods
29 6 days Jan. 21–26	Total 71.6	U. 62.5 F. 5.0 67.5	+ 4.1	6.78	U. 3.32 F. 2.54 5.86	+ 0.92	2.148	U. 0.787 F. 1.277 2.064	+ 0.084	9.24	U. 6.69 F. 1.67 8.36	+ 0.88	Control periods
30 3 days Jan. 27–29	Total 36.4	U. 33.3 F. 3.5 36.8	— 0.4	3.35	U. 1.71 F. 2.01 3.72	— 0.37	1.040	U. 0.437 F. 0.857 1.294	— 0.254	4.58	U. 3.37 F. 1.33 4.70	— 0.12	Control periods
Totals for 21 days	Total 258.1	240.0	+ 18.1	23.31	19.64	+ 3.67	7.472	7.180	+ 0.292	32.27	28.60	+ 3.67	Totals pre-operative periods
31 6 days Jan. 30–Feb. 4	Total 49.7	U. 42.3 F. 3.2 45.5	+ 4.2	8.83	U. 1.02 F. 1.86 2.88	+ 5.95	1.332	U. 0.442 F. 0.645 1.087	+ 0.245	8.03	U. 2.05 F. 1.05 3.10	+ 4.93	Operation January 30 liquid diet
32 3 days Feb. 5–7	Total 30.1	U. 31.1 F. 1.6 32.7	— 2.6	3.40	U. 0.16 F. 0.93 1.09	+ 2.31	0.571	U. 0.206 F. 0.322 0.528	+ 0.043	3.86	U. 2.52 F. 0.53 3.05	+ 0.81	Semi-solid diet
33 6 days Feb. 8–13	Total 78.7	U. 58.5 F. 4.5 63.0	+ 15.7	7.03	U. 0.35 F. 1.54 1.89	+ 5.14	2.116	U. 0.614 F. 1.221 1.835	+ 0.281	9.63	U. 4.59 F. 1.60 6.19	+ 3.44	Pre-operative diet
34 6 days Feb. 14–19	Total 76.5	U. 50.9 F. 5.7 56.6	+ 19.9	6.87	U. 0.34 F. 1.19 1.53	+ 5.34	2.000	U. 0.548 F. 1.064 1.612	+ 0.388	9.14	U. 4.11 F. 1.46 5.57	+ 3.57	Pre-operative diet
35 6 days Feb. 20–25	Total 74.9	U. 61.1 F. 5.7 66.8	+ 8.1	6.76	U. 0.28 F. 1.62 1.90	+ 4.86	1.976	U. 0.671 F. 1.172 1.843	+ 0.133	9.16	U. 4.98 F. 1.59 6.57	+ 2.59	Pre-operative diet
Totals for 27 days	Total 309.9	264.6	+ 45.3	32.89	9.29	+ 23.60	7.995	6.905	+ 1.090	39.82	24.48	+ 15.34	Totals for post-operative periods

Legend: U = excretion in urine; F = excretion in feces; + = retention; — = loss.

TABLE 3

Balances of sodium, potassium and chlorine

PERIOD	WEIGHT,[1] KG.	SODIUM, GM.			POTASSIUM, GM.			CHLORINE, GM.			COMMENT
		Intake	Output	Balance	Intake	Output	Balance	Intake	Output	Balance	
27 6 days Jan. 9–14	51.010 (+ 570)	NaCl 7.08 Food 6.08 Total 13.16	U. 12.52 F. 0.04 12.56	+ 0.60	29.00	U. 25.10 F. 1.66 26.76	+ 2.24	10.92 11.44 22.36	U. 21.60 F. 0.09 21.69	+ 0.67	Control periods
28 6 days Jan. 15–20	51.580 (+ 820)	NaCl 7.08 Food 5.49 Total 12.57	U. 10.15 F. 0.04 10.19	+ 2.38	28.16	U. 22.69 F. 1.85 24.54	+ 3.62	10.92 10.68 21.60	U. 19.32 F. 0.12 19.44	+ 2.16	Control periods
29 6 days Jan. 21–26	52.400 (+ 20)	NaCl 7.08 Food 5.49 Total 12.57	U. 12.78 F. 0.06 12.84	— 0.27	27.96	U. 25.85 F. 1.91 27.76	+ 0.20	10.92 11.53 22.45	U. 21.31 F. 0.21 21.52	+ 0.93	Control periods
30 3 days Jan. 27–29	52.420 (— 80)	NaCl 3.54 Food 2.81 Total 6.35	U. 6.98 F. 0.45 7.43	— 1.08	14.22	U. 12.63 F. 1.98 14.61	— 0.39	5.46 5.30 10.76	U. 12.21 F. 0.65 12.86	— 2.10	Control periods
Totals for 21 days			44.65	43.02 + 1.63	99.34	93.67	+ 5.67	77.17	75.51	+ 1.66	Totals for pre-operative periods
31 6 days Jan. 30–Feb. 4	52.340 (+ 1310)	NaCl 3.53 Food 6.61 Total 10.14	U. 5.26 F. 0.12 5.38	+ 4.76	17.36	U. 9.60 F. 1.77 11.37	+ 5.99	6.77 11.91 18.68	U. 11.53 F. 0.52 12.05	+ 6.63	Operation January 30 liquid diet
32 3 days Feb. 5–7	53.650 (— 770)	NaCl 3.54 Food 2.92 Total 6.46	U. 5.17 F. 0.06 5.23	+ 1.23	7.92	U 9.58 F. 0.89 10.47	— 2.55	5.46 5.03 10.49	U. 7.66 F. 0.26 7.92	+ 2.57	Semi-solid diet
33 6 days Feb. 8–13	52.880 (+ 1220)	NaCl 7.08 Food 5.54 Total 12.62	U. 10.24 F. 0.21 10.45	+ 2.17	28.92	U. 24.90 F. 1.80 26.70	+ 2.22	10.92 10.40 21.32	U. 19.22 F. 0.23 19.45	+ 1.87	Pre-operative diet
34 6 days Feb. 14–19	54.100 (— 560)	NaCl 6.69 Food 5.20 Total 11.89	U. 12.36 F. 0.39 12.75	— 0.86	27.08	U. 21.45 F. 2.43 23.88	+ 3.20	10.32 10.71 21.03	U. 21.91 F. 0.26 22.17	— 1.14	Pre-operative diet
35 6 days Feb. 20–25	53.540 (+ 440) 53.980	NaCl 7.08 Food 5.88 Total 12.96	U. 11.39 F. 0.44 11.83	+ 1.13	28.12	U. 25.30 F. 2.88 28.13	— 0.01	10.92 12.20 23.12	U. 20.87 F. 0.34 21.21	+ 1.91	Pre-operative diet
Totals for 27 days			54.07	45.64 8.43	109.40	100.55	+ 8.85	94.64	82.80	+ 11.84	Totals for post-operative periods

[1] Weights are given for the first day of each metabolic period, except in period 35 where the final weight recorded is for the close of this period. Values in parenthesis give the gain or loss of weight in grams which occurred in the period.

Legend: U = excretion in urine; F = excretion in feces; + = retention; — = loss.

When the moderately high intake of calcium and phosphorus is considered, the excretion of each in the feces during the pre-operative periods is seen to have been distinctly less than

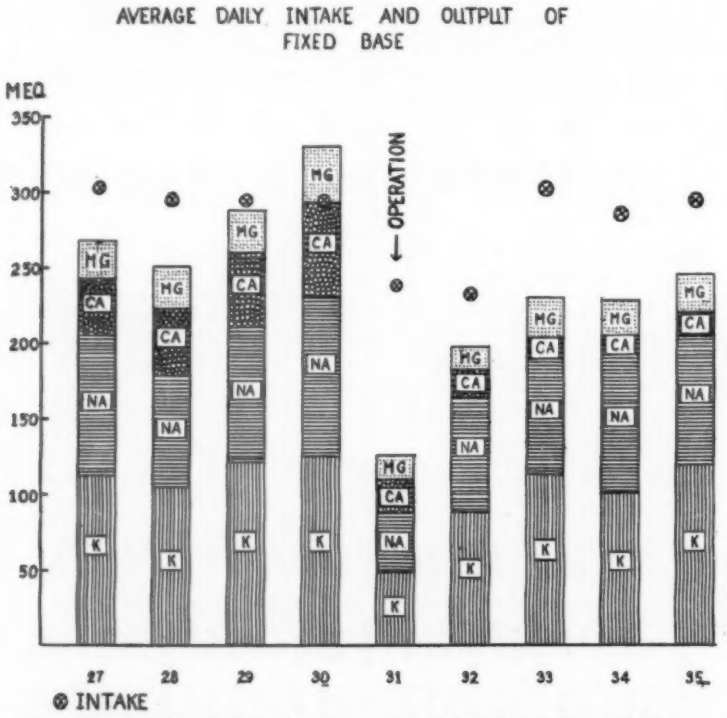

Fig. 1 The height of each column represents the total excretion of fixed base. Individual components are indicated by the appropriate chemical symbol. 'Intake' refers to the total amount amount of fixed base furnished by diet and solution of sodium chloride. Numerals at the foot of each column show the period for which the average has been computed. Operation was performed on the first day of period 31.

normal. Following the operation, there was little or no change in the the amount of fecal phosphorus, while the excretion of calcium in the feces actually appeared to decrease slightly.

A very marked retention of both calcium and phosphorus occurred post-operatively and continued until the experiment was terminated. The total retentions were roughly in the proportions in which these elements occur in bone. However, this was not necessarily the case in individual periods. For example, much more phosphorus was retained in the operative

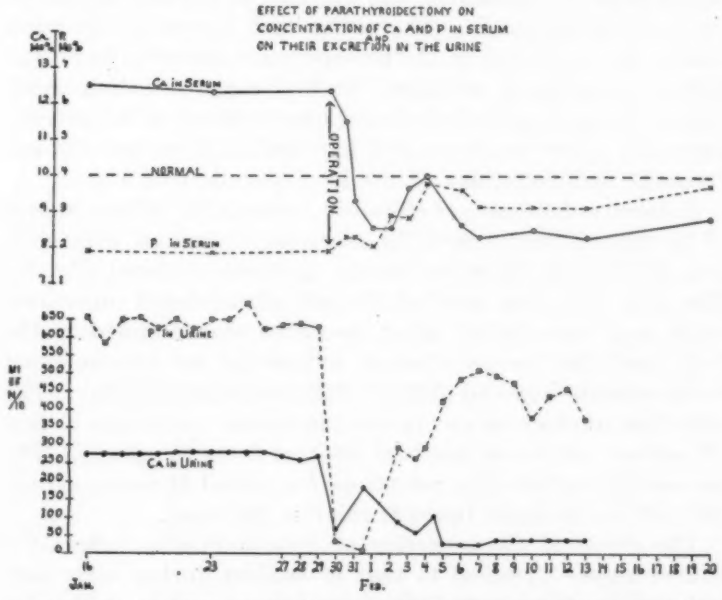

Fig. 2 The immediate effects of removal of the parathyroid tumor on calcium and phosphorus of blood and urine are shown in the chart. Note the almost complete disappearance of phosphorus from the urine without significant increase in the concentration of inorganic phosphorus in the serum.

period (period 31) than was needed to combine with the calcium simultaneously retained. Attention has again been directed to this fact in the subsequent discussion of the metabolism of sodium and potassium.

Magnesium. The effect of parathyroidectomy (removal of a parathyroid tumor) on the metabolism of magnesium appears to be of the same nature as its effect on the metabolism

of calcium and phosphorus. Distinctly more magnesium was retained following operation than before it, and the excretion of magnesium in the urine was moderately decreased in post-operative periods. Our observations, therefore, confirm those reported by Bulger and Gausmann ('33).

Nitrogen. Equilibrium, which appears to have been established prior to operation, was maintained during the first 9 days of convalescence. Thereafter with return to the more liberal diet provided in the pre-operative periods, there was definite retention of nitrogen. Such nitrogen retention seems logical during a period of clinical improvement of the patient, especially since weakness and hypotonia of the muscles are frequent manifestations of the hyperparathyroid state.

Sodium, potassium and chlorine. Immediately after removal of the parathyroid tumor, the excretion of sodium, potassium and chlorine in the urine became markedly reduced (fig. 3). The data show that most of the salt administered subcutaneously and immediately after operation was retained in the body, and that the excretion of sodium did not become equal to the amount ingested until the fifth post-operative day. The excretion of chlorine was in most instances equivalent to that of sodium and hence has been omitted from the graph. The net sodium and chlorine retentions for period 31 were: sodium 207 milli-equivalents (m.eq.); chlorine 187 m.eq.

The curve of the excretion of potassium also followed a course almost identical to that of sodium during early convalescence. Since very little potassium was given on the day of operation, a small loss of potassium occurred on this day. However, on subsequent days with the level of potassium intake reduced somewhat below the preoperative level, there was marked retention of potassium. The net balance for the period (period 31) showed a retention of 152 m.eq. of potassium. Whether the retention of potassium in period 31 was related in any manner to the apparently excessive retention of phosphorus in this period is a matter for conjecture. Should all of the calcium deposited in the period have been in the form of tricalcium phosphate, there yet remained 108 m.eq. of

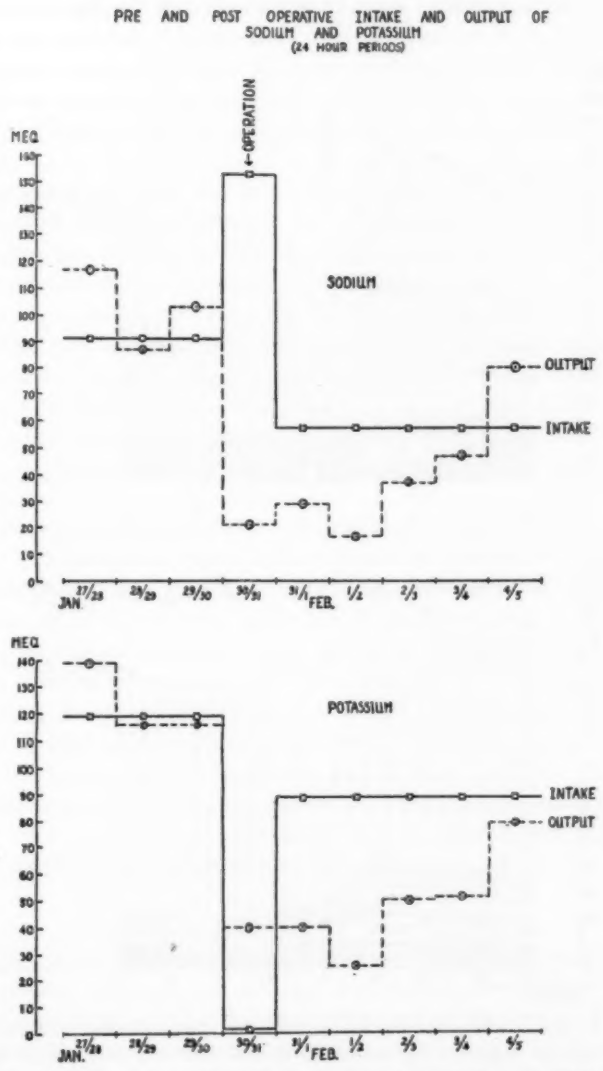

Fig. 3 Operation was performed on the morning of January 30th. Note retention of sodium and potassium on this and subsequent days.

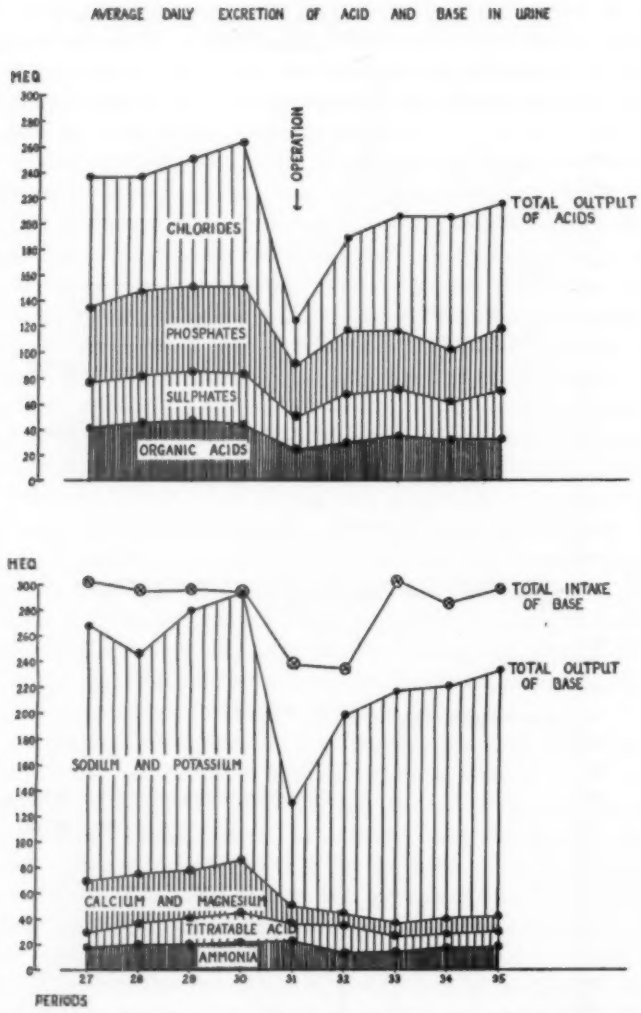

Fig. 4 The acid-base balance of the urine has been computed and illustrated in the manner suggested by Gamble, Ross and Tisdall ('23), except that the urinary excretion of carbonates has not been estimated. The very marked decrease in the excretion of acid and base in the urine during period 31 was in part due to decreased intake, and in part due to retention of calcium, magnesium, sodium, potassium, phosphorus and chlorine in the body.

phosphorus (calculated at a valence of 1.8) which might have been neutralized by potassium. As implied in the introductory remarks, this suggestion was brought forward by Greenwald ('13) to explain the retention of base (other than calcium) which followed the operation of thyreoparathyroidectomy in dogs.

Excretion of acid and base in the urine. The average daily excretion of acid and base in the urine in each period is shown in figure 4. The data have been arranged in such a manner that the areas between lines are roughly proportional to the several components comprising the total excretion. The factors which conserve fixed base (ammonia and titratable acid) have been included in the diagram which represents the total excretion of base (Gamble, Ross and Tisdall, '23) and hence the latter should exactly balance the excretion of acid. This appears to be the case within limitations imposed by methods.

The major changes occurred in period 31 and these, as noted above, followed in part as the result of decreased intake of mineral elements in the food and in part because of marked retention of fixed base, chlorine and phosphorus. With return to the preoperative diet (periods 33, 34 and 35), aside from decreased excretion of calcium, phosphorus, and magnesium, there was noted a very small decrease in the excretion of ammonia, a somewhat greater decrease in the excretion of titratable acid and a slight decrease in the excretion of organic acid. The decrease in the excretion of ammonia and titratable acid was probably due to diminished excretion of phosphates and organic acids.

DISCUSSION

We are of the opinion that the moderate and apparently temporary disturbance of salt and water metabolism which has been depicted for the operative period was part of a general readjustment of electrolyte balance made necessary by the removal of the parathyroid tumor. This position is difficult to maintain, unless it can be shown that the general effects

of the operative procedure and of the unavoidable departures from the routine of the fore periods had no significant relation to the results obtained or were actually inimical to them. Perhaps the most obvious fallacy in the usual method of estimating the balances of sodium and chlorine is the well-known fact that variable amounts of salt may be excreted in sweat (Cramer, 1890; Atchley et al., '33) and that such losses remain unrecorded. Moreover, loss of water and electrolytes through bleeding introduces another source of error which should not be disregarded (Coller and Maddock, '32). It is impossible to answer the first objection in an entirely satisfactory manner without measurement of excretion by the skin. Observations have, however, been made of the concentration of sodium in sweat (Bassett and Warren, '33) under conditions which undoubtedly place this value too high. A simple computation based on these data shows that one would be forced to assume a secretion of about 1100 ml. of sweat on the day of operation if the apparent retention of 132 m.eq. of sodium during the 24 hours involved were due entirely to skin losses. It is extremely improbable that such an amount of sweating could have occurred and yet have remained undetected. Bleeding as a source of error can be excluded with certainty, since excellent hemostasis was obtained.

The effect of anesthesia (avertin plus nitrous oxide and oxygen) is less readily evaluated, but presumably favors dehydration (Coller and Maddock, '32) and hence loss of electrolytes corresponding to the decrease in volume of body water (Gamble, '29). Recent studies, however, indicate that minor degrees of dehydration are not necessarily attended by disturbance of salt balance (Wiley and Wiley, '33).

Reduction in expenditure of energy (since there was no fever) must have occurred in the operative period due to the enforced rest in bed. A reduction in caloric intake also occurred at this time due to the single day of fasting at the beginning of the period and the slight decrease in the intake of food on the remaining days. When an increment of 15

per cent was added to the basal metabolism of the patient to allow for movements in bed and to cover the specific dynamic action of the food ingested, a rough approximation of the energy expended in the operative period was obtained. The estimated production of heat, 9400 calories, was found to be nearly the same as that of the caloric intake, which for the period amounted to 9700 calories. Overfeeding, then, could hardly have been a factor in the gain in weight or retention of salt. Furthermore, it is to be recalled that the retention of sodium and potassium occurred at a time when their levels of intake had been reduced and hence the balance seems to have been opposite in direction to that which might have been expected.

Exclusion of a possible slight disturbance of thyroid function, incident to manipulation of the gland at operation, as a cause of retention of base seems justifiable. In support of this contention we cite again the quite dissimilar experiments of Greenwald and of Albright and collaborators where the only common factor which could have been held responsible for retention of base was the sudden reduction in the amount of parathormone made available to the body.

The response of our patient by retention of a large part of the salt administered as saline solution on the day of operation was qualitatively similar to that of an edematous individual who ingests a solution of sodium chloride (Schittenhelm and Schlecht, '19) or to that of a normal subject whose intake of salt has been restricted (Vallery-Radot, '18; Veil, '23; Loeb, Atchley, Benedict and Driscoll, '32; Wiley, Wiley and Waller, '33). In the present instance no rigorous restriction of salt preceded operation; the daily intake of sodium chloride averaged 91 m.eq. in preoperative control periods and this amount was increased to 152 m.eq. on the day of operation. Subsequently it was reduced to 57 m.eq. for the remainder of period 31. Unfortunately, differences in the mode of administration and in the total quantity of salt given do not permit any close analogy with previous work.

Approximately half of the potassium retained in period 31 was re-excreted in period 32, but there was no loss of sodium

or chlorine. A decrease in weight accompanied the loss of potassium. Both of these effects seem to have been contingent upon a further reduction in the caloric intake, especially since the activity of the patient had been considerably increased by allowing her to get out of bed and walk about the room. With resumption of the diet of the fore periods equilibrium was again slowly attained, but only after retention of more than enough potassium to balance losses sustained in period 32.

SUMMARY

Evidence has been presented which indicates that the extirpation of a parathyroid tumor is accompanied by a temporary derangement of the metabolism of salt and water. It was found that:

1. In addition to the well-recognized alteration in the concentrations of calcium and inorganic phosphorus in the serum and their retention in the body, there was also retention of significant amounts of sodium, potassium, magnesium and chlorine.

2. The retention of the latter group of elements since accompanied by a definite increase in weight strongly suggests an increase in the volume of body water as part of the mechanism involved in readjustment.

3. The temporary nature of the disturbance of salt and water balance was attested by the fact that approximate equilibrium between intake and outgo of sodium and potassium occurred on the fifth or sixth post-operative day and hence coincided with the time at which the concentrations of calcium and inorganic phosphorus of the blood and their excretion in the urine were definitely established at normal levels.

4. While retention of the principal solutes of body fluids was found to follow removal of a parathyroid tumor, an approximately quantitative measurement of the exchanges of sodium, potassium and chlorine did not support the view that alterations in the metabolism of these elements is of primary importance in hyperparathyroidism.

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A STATISTICAL STUDY OF THE METABOLISM OF THE FASTING ALBINO RAT

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ONE FIGURE

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During the course of the investigation of certain polyatomic alcohols and their anhydrides as food stuffs for the albino rat, the authors (Carr et al., '33; Carr and Krantz, '34; Carr et al., '34) determined the respiratory quotient and heat production of nearly 100 fasting animals. These data were thought to be of sufficient interest to assemble in one communication.

Similar studies generally on a much smaller number of animals have been conducted by Benedict and MacLeod ('28) Wesson ('30) and Cori and Cori ('26).

EXPERIMENTAL

With certain minor modifications, Haldane's open circuit (1892) apparatus was employed. Male rats were used in these experiments and prior to the determination were kept at 28°C. and fed a balanced diet. They were fasted 48 hours prior to the experiment. The animal was then placed in the respiratory chamber.

Air was blown at the rate of 2 liters a minute through two scrubbing bottles of moist soda lime no. 4 mesh and one bottle of calcium chloride, and then through pumice, which had been thrust into sulphuric acid at bright red heat according to the Haldane technic. This air passed over the animal for 20 to

30 minutes. After this period the metabolism chamber was closed, the air entrapped within having been adjusted to 28°C. ± 0.5°. The metabolism chamber and animal were weighed on a balance with a load capacity of 2000 gm. and a sensitivity of 1 mg. Third decimal place weighings were employed.

The metabolism chamber was then inserted in the air line. The exit tube was connected with a series of absorption bottles containing sulphuric acid and pumice for water absorption and a soda-lime bottle and sulphuric acid pumice bottle for the absorption of carbon dioxide. Check absorption bottles for water and carbon dioxide were kept in the chain and weighed with each determination, as a check on the efficiency of the apparatus. The intake air was checked by blank runs before each series of six determinations. In this set up, the efficiency of the water absorption apparatus is worthy of special comment. After thirty runs (of 2½ hours each) the check water absorption bottle did not increase in weight significantly, i.e., ± 0.001 gm. The soda-lime required remoistening or replenishing after ten or twelve runs. Each absorption bottle for water contained about 600 gm. of the pumice-sulphuric acid mixture: those containing soda-lime had slightly less than 500 gm. of that substance in each. Five hundred cubic centimeter Woulff bottles were employed, the intake tubes to these bottles passed through the central opening in the bottle. All stoppers and rubber connections were closed with sealing wax.

No correction was made for the nitrogen content of the urine of the animals as did Cori and Cori ('26), who showed that approximately 90 per cent of the metabolism in a fasting rat is fat oxidation. The determinations were conducted between 10 A.M. and 4 P.M., according to the suggestion of Horst et al. ('34). The surface area was calculated by the formula set forth by Diack ('30), $S = 7.64 \times W^{2/3}$ for fasted rats.

DISCUSSION OF RESULTS

The data given in the graph are obtained from rats that are apparently normal. Occasionally an animal selected for a metabolism experiment was found to have an exceptionally

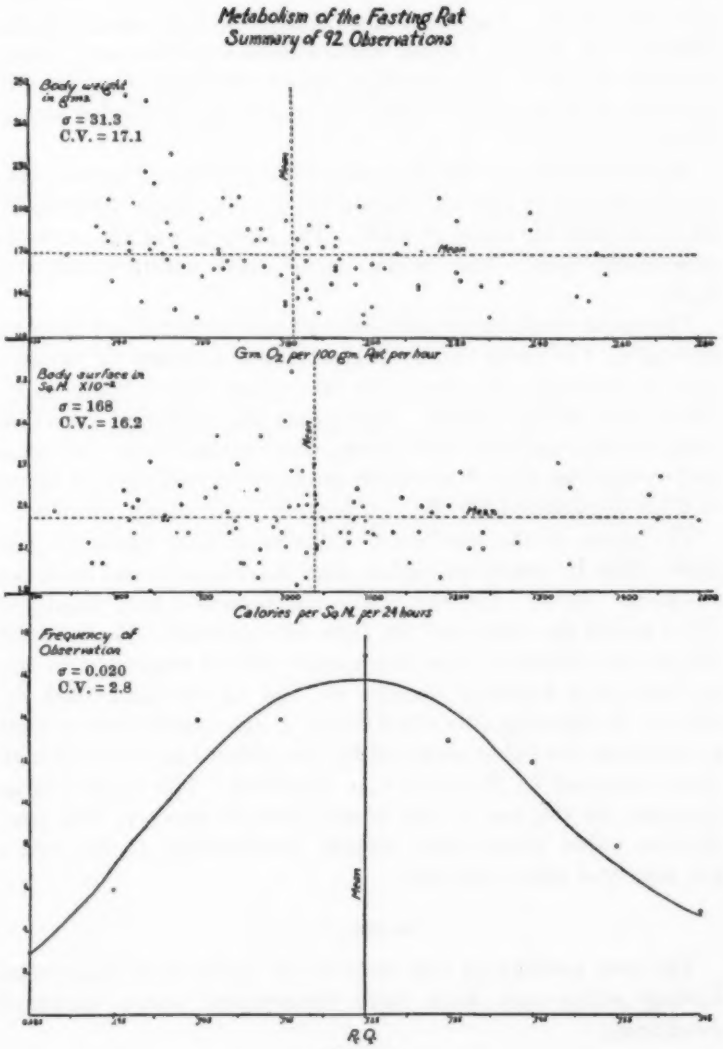
Metabolism of the Fasting Rat
Summary of 92 Observations
Body weight in gms.
σ = 31.3
C.V. = 17.1
Mean
Mean
Gm. O2 per 100 gm. Rat per hour
Body surface in Sq.M. X10⁻²
σ = 168
C.V. = 16.2
Mean
Mean
Calories per Sq.M. per 24 hours
Frequency of Observation
σ = 0.020
C.V. = 2.8
Mean
R.Q.

Figure 1

high or exceptionally low respiratory quotient; such animals were discarded. Cori and Cori ('27) have pointed out the influence of season, ketosis and infection on the respiratory quotient of rats. The average values obtained then are for apparently normal rats under very carefully controlled conditions.

An examination of the frequency distribution of the respiratory quotients of the rats shows the median value practically identical with the mean of 0.725. The skewness of the curve is practically zero. The modal series falls within 0.720 and 0.729.

The mean respiratory quotient was not corrected for urinary nitrogen. Cori and Cori ('26) set forth a group of twenty-four respiratory quotients for the albino rat. Their mean value was 0.711 ± 0.005. Accepting the urinary excretion value of Cori and Cori as 3.18 mg. per 100 gm. of rat per hour, and correcting the respiratory quotient accordingly, a mean of 0.708 is obtained for the non-protein respiratory quotient.

The mean of the number of calories of heat production is 1040. This is somewhat higher than the values found by other workers. At 28° for the white rat Benedict and MacLeod ('28) found the value not far from 880 calories. These lower values are obtained when the factor 9.13 is employed in the surface area formula instead of 7.64 as recommended by Diack. Employing this older value in the calculation of heat production, the value obtained by the authors agrees well with those obtained by Benedict and MacLeod. The higher value obtained by the use of the Diack formula gives a heat production value which more closely corresponds to the value for man and other animals.

SUMMARY

The heat production and respiratory quotient of ninety-two fasting white rats have been determined under standard conditions.

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THE VITAMIN CONTENT OF SULTANINA (THOMPSON SEEDLESS) GRAPES AND RAISINS

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In pursuance of a research begun in 1927 upon the vitamins of dried fruits, Thompson seedless grapes of the crop of 1931 and raisins prepared from them were preserved for examination as to their vitamin values.

Only a few reports of such studies upon grapes and raisins have appeared. Dutcher and Outhouse ('23) examined Thompson seedless, loose Muscat and seeded Muscat raisins with negative results as to vitamins A and C, but positive as to vitamin B, then still undifferentiated. They made no tests upon corresponding fresh grapes. Randoin ('28) reported low vitamin C content in grape juice and wines, little antineuritic B, but appreciable amounts of antiberiberi B. The nomenclature is confusing, but in the light of later work it would appear that the former is vitamin G(B_2) and the latter B(B_1). Daniel and Munsell ('32) examined Thompson seedless, Malaga grapes and two brands of commercial grape juice for vitamins A, B, C and G. They did not include raisins in their study.

As has been the custom in our researches on dried fruit the Thompson seedless grapes were picked fresh, placed in cans, evacuated twice and the vacuum released with nitrogen then kept stored at 0°C. Five lots of raisins were prepared

from the similar fruit by methods outlined in table 1. The so-called soda-dipping consisted of dipping the fruit for about 30 seconds in lye held at 180°F. and then spreading on trays. Two lots, HS4 and HS6, were exposed to the fumes of burning sulfur for 5 hours before being dried.

Experimental methods. The technic employed for some years in this laboratory and previously described was used in the tests for vitamins A and C (Morgan, Field and Nichols, '31). For vitamin B(B_1) the method of Chase and Sherman

TABLE 1

Preparation and composition of Sultanina (Thompson seedless) grapes and raisins

LOT	LYE DIPPING	SULFURING	METHOD OF DRYING	MOISTURE	SULFUR DIOXIDE	pH	SHRINKAGE RATIO
	seconds	*hours*		*per cent*	*p.p.m.*		
HS1	..	..	Fresh grapes not dried, kept frozen	75.3	....	4.00	1:1
F.G.	..	..	Fresh grapes from market	73–76			1:1
HS2	..	..	7 days in sun, stacked after 16 days, boxed after 48 days	16.6	....	4.00	3.34:1
HS3	30	..	Same as HS2	15.8	....	4.00	3.41:1
HS4	30	5	Same as HS2	15.8	1350	3.90	3.41:1
HS5	30	..	Dehydrated at 120–160°F. for 38 hours, relative humidity 65–25 per cent, air velocity 500–700 feet per minute, boxed after 4 days	12.0	....	3.95	3.56:1
HS6	30	5	Same as HS5	11.8	1640	4.00	3.57:1

('31) was used with rats and the prophylactic method of Salmon ('27) with pigeons. Such tests as were made for vitamin G (B_2) were made by a modification of the method of Bourquin and Sherman ('31).

VITAMIN B ASSAY

Rat technic. The basal diet for rats consisted of purified casein 18, Osborn and Mendel salts 4, autoclaved yeast 15, butterfat 8, cod liver oil 2, corn starch 53. Animals receiving this diet and 0.05 cc. daily of a rice polish concentrate made according to the method of Evans and Lepkovsky ('31) were

able to grow practically at the normal rate. The rats were placed on the basal diet at 28 days of age and after 2 or 3 weeks, when their weights plateaued, were fed weighed amounts of the fruit for 56 days.

Pigeon technic. Young but mature male pigeons were confined in small individual cages and fed by hand daily 8 gm. of baked polished rice and 8 gm. of an autoclaved mash made up of casein 19, polished rice 68, agar 2, calcium phosphate 0.5, calcium carbonate 1, sodium chloride 0.5, autoclaved yeast 6, cod liver oil 3. Enough vitamin B containing food must be given along with this ration to maintain weight and prevent disgorging, paralysis and other symptoms of polyneuritis. The pigeons were first fed the basal diet alone until they showed some of these symptoms and were then given varying doses of wheat germ in order to determine their vitamin requirement. After 2 to 4 weeks on a given dose if no untoward symptoms appeared, the dose was decreased by 10 per cent and given for another 2 to 4 weeks. The first symptom observed, usually within 3 to 7 days after the dose was lowered below the necessary minimum, was disgorging of the food. When this occurred on 2 or more successive days along with weight loss, it was assumed that the preceding dose, 10 per cent larger, had been the minimum. Restandardizing of the birds was found to be necessary every 4 or 5 months, but the same birds could be used again and again.

As has been shown by Cowgill, Deuel, Smith, Klotz and Beard ('32), a rough relationship was found to exist between the minimum pigeon dose and the rat maintenance unit. In our colony the pigeon daily dose appears to be six or seven times the daily dose required to produce 25 gm. gain in 8 weeks in young rats. The formula of Cowgill and co-workers applied to rats and pigeons of the weights used in our experiments gives a lower ratio, about 4.5. In terms of the International vitamin B_1 standard adsorbate, the pigeon dose was 50 mg., whereas the rat dose of 10 mg. produced only 34 gm. gain in 8 weeks. There is some uncertainty involved in attempting such comparisons of maintenance in adult birds with subnormal growth in young rats.

As is shown in table 2 the fresh frozen grapes were distinctly inferior in vitamin B value to similar fresh grapes purchased two or three times a week in the local market. Apparently, the removal of air from the cans containing the

TABLE 2

Vitamin B(B_1) content of Sultanina (Thompson seedless) grapes and raisins by rat assay

FRUIT	LOT NO.	AMOUNT FED DAILY	EQUIVALENT IN FRESH FRUIT	NUMBER OF RATS	INITIAL (DEPLETED) WEIGHT	FINAL WEIGHT	GAIN OR LOSS	PERIOD	RETENTION OF VITAMIN B OF FRESH GRAPES
		gm.	*gm.*		*gm.*	*gm.*	*gm.*	*days*	*per cent*
Fresh (frozen) seedless Thompson grapes	HS1	1.0	1.0	4	81	52	−29	12	37
		1.5	1.5	8	62	63	1	38	
		2.0	2.0	2	77	71	−6	49	
		3.0	3.0	3	75	84	9	56	
Fresh seedless Thompson grapes from market	F.G.	3.0	3.0	11	59	83	24	56	100
		4.0	4.0	4	58	91	33	56	
		5.0	5.0	6	66	113	47	56	
Sun-dried raisins, natural	HS2	1.5	5.0	5	57	85	28	56	60
		2.0	6.7	6	73	104	31	56	
		2.5	8.3	9	74	116	42	56	
Soda-dipped sun-dried raisins	HS3	1.0	3.4	5	71	92	21	56	94
		1.5	5.1	10	56	104	48	56	
		2.0	6.8	7	47	105	58	56	
Soda-dipped dehydrated raisins	HS5	1.5	5.3	11	63	87	24	56	56
		2.0	7.1	10	54	84	30	56	
Soda-dipped dehydrated sulfured raisins	HS6	1.5	5.3	3	61	44	−17	22	0
		2.0	7.1	10	54	43	−21	28	
		3.0	10.7	10	67	52	−15	30	
None				23	50	46	−4	25	
International standard		0.01		12	65	99	34	56	

frozen grapes is not protective of this vitamin as it is usually of A and C. We have no explanation of this deterioration which was also observed, contrary to the usual experience, in the case of vitamin A and possibly also of vitamin C. Some

enzyme active under the conditions of storage described may be postulated. The fresh grapes contain 1 Sherman unit in 3 gm. or 1 International unit in 4 gm.

The best retention of the original vitamin B of the grapes was shown by the soda-dipped sun-dried raisins, HS4, and about equal retention 56 and 60 per cent by the dehydrated dipped raisins, HS5, and the natural undipped sun-dried fruit, HS2. Possibly the soda dipping improved digestibility of HS3 somewhat over that of HS2.

Almost complete loss of antineuritic value was seen in HS6 the dehydrated sulfured raisins. This was confirmed by pigeon tests also upon HS4, sun-dried sulfured raisins, and later by comparison of sulfured and unsulfured peaches, figs and rice polish concentrate. Whether this destructive action concerns only B(B_1) or another factor also, perhaps B4, cannot be stated at this time. Certainly, the effect of the sulfur dioxide is more disastrous in the rat than in the pigeon tests.

An experiment designed to test the effect of SO_2 on the vitamin more definitely was recently carried out.[1] The rice polish concentrate already mentioned was exposed to the action of washed sulfur dioxide gas by bubbling the gas from a commercial cylinder through the diluted extract for 1 hour at room temperature. The retained SO_2 was largely removed by evacuation from the concentrate, but a measurable amount remained as may be seen in table 3. Attempts were made to recover the vitamin B activity of the SO_2-treated extract by bubbling CO_2 through it for 3 hours and by treatment with barium chloride with subsequent removal of the barium with sodium sulfate.

As may be seen in table 3, none of the three types of treated extract appeared to retain antineuritic activity in any degree.

A few tests upon pigeons with the SO_2-treated extract have likewise proved negative, although by sufficiently increasing the dose it has been possible to defer or entirely prevent polyneuritis. Apparently, the vitamin B_1 destruction is not com-

[1] The help of Elizabeth Ludlow Fawkes in this part of the experiment is gratefully acknowledged.

plete, although another factor, possibly B_4, needed by rats and not by pigeons, may be entirely destroyed by the treatment.

It is interesting to speculate upon the mechanism of this action of sulfur dioxide. The vitamin may be reduced, hydrolyzed or a sulfite product formed. The theory that hydrolysis by sulfurous acid may occur seems improbable in view of the well-known resistance of the vitamin to the action of even stronger acids. Since basic properties are usually ascribed

TABLE 3

Vitamin B value of rice polish extract as affected by sulfur dioxide

DAILY AMOUNT FED	TREATMENT	pH	SO_2	NUMBER OF RATS	WEIGHTS			PERIOD OF TEST
					Initial	Final	Loss or gain	
gm.			*p.p.m.*		*gm.*	*gm.*	*gm.*	*days*
0.025	Untreated extract	5.4		11	59	90	31	56
0.050				11	71	157	86	56
0.100				5	70	225	155	56
0.025 to 0.200	SO_2 treated	5.8	1570	12	78	56	—22	20
0.1 and 0.2	SO_2 treated and Ba ppt or CO_2 saturated	6.2	740 1380	10	76	49	—27	24
	No vitamin B			28	53	46	—7	22
0.010	International standard			12	65	99	34	56

to vitamin B, an inactive sulfite may be postulated, but the failure of reactivation by barium treatment or carbon dioxide speaks against this.

There remains the reducing action of the SO_2. Kramár ('24) reported loss of antineuritic activity as resulting from drastic reduction of alcoholic extracts of yeast by aluminum amalgam in alkaline medium, but failure of such loss of activity when zinc-hydrochloric acid in acid medium was used as the reducing agent. Control experiments seemed to show that the alkali alone was not responsible for the destruction in the former case. Another significant finding is that of

Levene and Van der Hoeven ('25), who increased the potency of the yeast extract product obtained by preliminary precipitation with basic lead acetate by removal of the lead with sulfuric acid instead of hydrogen sulfide. Failure to remove the active substance from the precipitate when the latter reagent was used might be due to its destruction through reduction.

No other attempts at reduction of vitamin B have been found reported in the literature. The present study may be interpreted as evidence in favor of the easy destruction of the vitamin by reducing action. This is in interesting contrast with the easy destruction by oxidation of certain other vitamins, namely, A and C, and probably also, but in less degree, D and E.

In table 4 the detail of some of the observations upon pigeons is given and in table 5 a summary of the findings by this method. The results agree remarkably well with those obtained with rats, with the exception that the frozen fruit appeared undeteriorated. This may be due to the fact that these were the first tests made and that the grapes may have been as yet unaffected.

In any case the unsulfured raisins contained 1 Sherman unit in 1.0 to 1.5 gm. of fruit or 1 International unit in 1.5 to 2.0 gm. and a pigeon maintenance unit in 7 gm. The distinctly lower values seen by Daniel and Munsell ('32) in their Thompson seedless grapes resemble our figures for the frozen stored grapes, which doubtless their samples, purchased in Washington, D. C., after storage in transit from California, resembled more than they did the freshly gathered grapes from the Berkeley market.

VITAMIN A ASSAY

Again, when the vitamin value of the frozen grapes was tested it was found that a very considerable decrease, about 50 per cent, had occurred in frozen storage. This was somewhat surprising because the fruit was kept in air-evacuated cans. The change which occurred was apparently anaerobic in

TABLE 4

Pigeon tests for vitamin B assay of raisins and grapes

NUMBER OF BIRD	PERIOD	WEIGHT OF BIRD	MATERIAL FED	DAILY DOSE	REMARKS
	days	*gm.*		*gm.*	
15	29	404–453	HS3	7.0	Normal
	16	451–445	HS3	6.3	
	12	442–438	HS3	6.7	Disgorged 5 times
	9	427–445	HS3	7.0	Normal
	21	450–393	HS3	6.7	Disgorged 5 times, polyneuritic
	31	391–416	HS2	8.0	Normal
	5	416–418	HS2	7.0	Disgorged 3 times
	19	403–409	HS6	8.0 and 0.3 gm. wheat germ	Disgorged 11 times
	24	409–431	HS6	8.0 and 0.4 gm. wheat germ	Disgorged 4 times, polyneuritic on 24th day. Given 3 gm. yeast
	37	421–446	HS6	8.0 and 0.5 gm. wheat germ	Normal
	27	446–441	HS6	8.0 and 0.4 gm. wheat germ	Disgorged on 19th and 27th days
3	60	435–369	Fresh frozen seedless Thompson grapes (HS1)	4–11	Disgorged, lost weight. Insufficient protection
	30	356–382	Fresh frozen seedless Thompson grapes (HS1)	16.0	Normal
	25	382–404	Fresh frozen seedless Thompson grapes (HS1)	14.4	Normal
	30	377–395	Fresh market grapes	15.8	Disgorged 5 times
	14	377–382	Fresh market grapes	17.0	Normal
2	33	452–404	HS1	10–17	Disgorged constantly
	36	394–400	HS1	18.0	Nearly normal
	3		HS1	16.2	Disgorged
	8	396–392	HS6	8 and 0.4 gm. wheat germ	Constant disgorging
	30	392–409	HS6	8 and 0.5 gm. wheat germ	Normal

TABLE 4—*Continued*

NUMBER OF BIRD	PERIOD	WEIGHT OF BIRD	MATERIAL FED	DAILY DOSE	REMARKS
	days	*gm.*		*gm.*	
	10	412–398	HS6	8 and 0.4 gm. wheat germ	Disgorged
14	19	401–431	HS2	7.0	Disgorged on 19th day
	43	424–403	HS2	8.0	Nearly normal
	7	401–376	HS2	7.0	Disgorged 3 times
	30	376–394	HS3	8.0	Normal
	30	392–398	HS3	7.0	Normal
	27	398–410	HS3	6.0	Disgorged after 27 days
16	30	406–385	HS4	7 to 13	Frequent disgorging
	8	343–356	HS4	7 and 0.45 gm. wheat germ	Normal
	30	351–394	HS6	8 and 0.45 gm. wheat germ	Normal
	30	394–393	HS6	6.4 and 0.45 gm. wheat germ	Normal
	8	388–387	HS6	6.4 and 0.3 gm. wheat germ	Constant disgorging
	36	371–404	HS5	8.0	Normal
	30	406–412	HS5	7.0	Normal
	7	413–408	HS5	6.0	Disgorged
10	30	386–397	HS5	8.0	Normal
	30	393–412	HS5	7.0	Normal
	10	416–415	HS5	6.0	Disgorged 3 times
	35	425–437	Wheat germ	0.70	Normal
	29	435–442	Wheat germ	0.63	Disgorged on 29th day

TABLE 5

Retention of vitamin B by Thompson seedless raisins as shown by pigeon assay

NUMBER OF BIRD	DAILY DOSE REQUIRED FOR MAINTENANCE				RETENTION OF VITAMIN B OF FRESH GRAPES
	Wheat germ	Fruit			
		Type	Amount	Equivalent in fresh fruit	
	gm.		*gm.*	*gm.*	*per cent*
10	0.7	HS5	7.0	25	64
16	0.9	HS5	7.0	25	64
15	0.8	HS3	7.0	24	66
14	0.9	HS3	7.0	24	66
14	0.9	HS2	8.0	27	59
15	0.8	HS2	7.0	23	69
15	0.8	HS6	8.0 and 0.5 gm. wheat germ	85	18
2	0.7	HS6	8.0 and 0.5 gm. wheat germ	85	18
16	0.9	HS6	6.4 and 0.45 gm. wheat germ	65	24
16	0.9	HS4	7.0 and 0.45 gm. wheat germ	68	23
2	0.7	HS1	18.0	18	
3	0.7	HS1	14.4	14	100
3	0.7	Fresh grapes	17.0	17	

character as it must also have been in the case of vitamin B. That it was progressive is evinced by the better response on 2 gm. doses of the frozen fruit carried out soon after the samples were procured than on 3 gm. doses made several months later. The fresh grapes appeared to contain 1 International unit (allowing 6 to 8 gm. gain per week for 8 weeks) in 2 gm. (table 6).

TABLE 6

Vitamin A content of Sultanina (Thompson seedless) grapes and raisins

FRUIT	LOT NO.	AMOUNT FED DAILY	EQUIVALENT IN FRESH FRUIT	NUMBER OF RATS	AVERAGE WEIGHTS		AVERAGE GAIN PER WEEK	EXPERIMENTAL PERIOD	WEIGHT YIELDING 1 UNIT (INTERNATIONAL) IN FRESH FRUIT EQUIVALENT	VITAMIN A RETENTION
					Initial	Final				
		gm.	*gm.*		*gm.*	*gm.*	*gm.*	*days*	*gm.*	*per cent*
Fresh grapes (frozen)	HS1	1.0	1.0	9	107	112	0.9	42		
		2.0	2.0	7	109	142	4.1	56	4.0	50
		3.0	3.0	3	120	148	3.5	54		
Fresh grapes	F.G.	3.0	3.0	4	103	187	10.5	56	2.0	100
		5.0	5.0	4	114	214	12.5	56		
Sun-dried raisins, natural	HS2	1.0	3.3	4	98	92		15		0
		2.0	6.7	6	107	92		18		
		3.0	10.0	13	98	91		24		
Soda-dipped sun-dried raisins	HS3	2.0	6.8	5	106	132	3.2	56		
		3.0	10.2	7	101	139	4.8	56	13.6	15
Soda-dipped sulfured sun-dried raisins	HS4	1.0	3.4	6	105	95		35		
		1.5	5.1	2	104	113	2.2	28		
		2.0	6.8	5	109	147	4.8	56	10.2	20
Soda-dipped dehydrated raisins	HS5	0.5	1.8	7	118	170	6.5	56	2.0	100
		1.0	3.5	9	112	196	10.5	56		
		1.5	5.3	5	111	201	11.2	56		
		2.0	7.1	7	95	199	13.0	56		
Soda-dipped sulfured dehydrated raisins	HS6	0.5	1.8	7	112	172	8.2	51	2.0	100
		1.0	3.5	12	111	207	12.0	56		
		1.5	5.3	6	97	205	13.5	56		
		2.0	7.1	8	110	206	12.0	56		
Carotene, International standard		*mg.* 0.001		12	104	156	6.5	56	*mg.* 0.001	

None of the sun-dried raisins, HS2, HS3 or HS4, showed more than a modicum of vitamin A. However, the two dehydrated specimens, HS5 and HS6, the latter sulfured, appeared to retain the full vitamin A content of the fresh fruit. This was the most decisive contrast so far seen between dehydrated and sun-dried fruit in this respect. The natural sun-dried raisins, HS2, were wholly lacking and the two soda-dipped products, HS3 and HS4, retained only 15 and 20 per cent of the vitamin of the fresh fruit. Such sensitivity to the exposure and heat of sun-drying was to be expected perhaps in a fruit so subject to enzyme changes.

The soda dipping appeared favorable, HS3 compared with HS2, perhaps because of better absorbability. The sulfuring appeared slightly favorable also, HS3 compared with HS4.

Again, the findings of Daniel and Munsell ('32) and Dutcher and Outhouse ('32) were less favorable than ours perhaps because of the losses in storage of the fresh grapes in the former case and the failure to test dehydrated raisins in the latter.

VITAMIN C ASSAY

The frozen grapes showed progressive loss of antiscorbutic property for, as shown in table 7, 20 gm. doses were more protective at first than 25 gm. doses later. Even fresh market grapes, however, did not afford full protection to the guinea pigs in 30 gm. daily doses. None of the raisin products were protective. It must be concluded that this variety of grape is not well endowed with vitamin C.

VITAMIN G CONTENT

An attempt was made to assess the vitamin G(B_2) content of these products, but without satisfactory conclusions. The daily doses fed were probably in most cases too small to give significant differences in the resulting growth. Fresh grapes in 2 gm. daily doses made an appreciable increase in the growth of rats fed the vitamin G-free basal diet as did 1 gm. doses of the raisins. The fruit appears rather poorly endowed

in this vitamin—a fact observed in the fresh grapes by Daniel and Munsell ('32). There is apparently about 1 unit (Bourquin-Sherman) of vitamin G in 2 gm. of the raisins, whether sulfured or unsulfured. A more decisive experiment on this point is yet to be undertaken.

TABLE 7

Vitamin C content of Sultanina (Thompson seedless) grapes and raisins

FRUIT	AMOUNT FED DAILY		NUMBER OF GUINEA PIGS	BODY WEIGHTS			PERIOD	SCURVY SCORE[1]
	Grams	Equivalent in fresh fruit		Initial	Final	Gain or loss		
		gm.		*gm.*	*gm.*	*gm.*	*days*	
HS1 (frozen grapes)	10, 15	10, 15	15	336	270	−66	47	10
	20	20	2	284	337	+53	60	1
	25	25	4	322	238	−84	41	9
Fresh grapes	30	30	3	318	372	+54	52	3
HS2 (natural raisins)	6, 8	20, 27	4	342	282	−60	53	5
HS3 (soda-dipped natural raisins)	4, 6	14, 20	6	329	244	−85	34	15
HS4 (sulfured natural raisins)	4–10	14–34	15	324	214	−110	39	12
HS5 (dehydrated raisins)	10	36	4	326	254	−72	38	14
HS6 (dehydrated sulfured raisins)	4–10	14–36	18	328	225	−103	52	11
	15	53	2	335	192	−143	60	9

[1] 0, no scurvy to 24, most severe scurvy.

DISCUSSION

Some interest of a practical as well as theoretical character attaches to the divergent effect of the sulfuring process upon the various vitamins. That such differences would be found in the effect of sulfur dioxide is to be expected, since each of the vitamins so far chemically identified is distinct in nature from all the others. Thus vitamins A and C are known to be readily oxidized labile molecules which, as has been found, might well be protected from destruction by a reducing substance such as sulfur dioxide. On the other hand, vitamin B

which is probably a base, not readily oxidized, but heat-labile, might be injured by the same substance. There is some previous evidence that this vitamin is readily reduced (Kramár, '24). Guha ('31) has stated that vitamin G(B_2) is not affected by sulfur dioxide and so far as our observations have gone this is true of the vitamin G of dried fruit.

There is no single rule which can be set forth as embodying the best practice in drying methods for all fruits or for all vitamins. Probably each fruit should be preserved in that fashion which conserves its best vitamin endowment. Thus peaches and apricots, which are excellent sources of A and C and probably poor sources of B, should be sulfured, but raisins and prunes which are good sources of B and poor sources, at least of C, should not be sulfured. Dehydration rather than sun-drying is advisable in addition if the vitamin A is to be adequately conserved.

SUMMARY

1. Seedless Thompson grapes when preserved by freezing storage, even after air evacuation, rapidly lose both vitamin A and B activities.

2. These grapes are rather well endowed with vitamin B when fresh and this endowment is largely retained in the natural sun-dried and soda-dipped raisins which contain 1 International (or about 1.4 Chase and Sherman) vitamin B unit in 1.5 to 2.0 gm. However, treatment of the fruit with sulfur dioxide previous to drying destroys much of the vitamin. Such destruction is more striking if shown by rat growth instead of by pigeon maintenance.

3. The fresh grapes are richer in vitamin A than has been hitherto supposed possible, because really fresh fruit has not previously been examined. The dehydrated raisins, both sulfured and unsulfured, retained this factor in full, 1 International unit in 0.5 gm. All the sun-dried raisins had lost most of the vitamin A activity.

4. The frozen grapes appeared to lose antiscorbutic activity in storage and even the fresh grapes were only poorly endowed

with vitamin C. Very little protective value could be ascribed to any of the raisins.

5. Vitamin G appears to be poorly represented in grapes and raisins, but conclusive experiments are not yet available.

6. Unsulfured soda-dipped dehydrated raisins made from Thompson seedless grapes retain practically all of the considerable vitamin A and B activity of the fresh grapes.

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THE VITAMIN CONTENT OF FIGS

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Figs of four varieties and three different crop years were used in the series of experiments here reported. The usual method (Morgan and Field, '29) developed for the study of the vitamin content of other dried fruits was followed in all cases. Fresh fruit was selected under known conditions, kept in frozen storage, and samples of similar fruit dried under varying conditions were examined as to vitamin activity along with the fresh. An attempt was thus made to discover the probable original vitamin content of the fruit as well as the effect upon this of the preserving methods used. No record of other vitamin studies upon figs has been found.

Kadota and Calimyrna figs were examined in 1929, Missions in 1930 and Kadotas and Adriatics in 1932. As shown in table 1, seventeen samples were prepared, six fresh and eleven dried, eight of the latter being sulfured. Only one sample, H12, was dehydrated, all others being sun-dried, as is the prevailing commercial fashion. Another factor of difference lay in the stage at which the fruit was picked. The fresh samples H9, H8, S5, F1 and F4 were picked from the trees at the stage suitable for consumption, and F7 was allowed to shrink to some extent before being picked. Certain of the dried products, H10, S6, S7, F8 and F9, were gathered after the natural drop from the trees, but the others, H11, H12, F2, F3, F5 and F6, were picked fresh and juicy and then subjected to the drying process. Only perfect fruits were used.

TABLE 1

Preparation and analysis of fig samples

VARIETY	YEAR	LOT NO.	PREPARATION	MOISTURE	SO_2	pH	DRYING RATIO
				per cent	*p.p.m.*		
Kadota	1929	H9	Fresh, ground, kept frozen in storage after evacuation and release of vacuum with nitrogen	68.2	....	4.6	1:1
Calimyrna	1929	H8	Fresh, prepared as was H9	65.2	....	4.3	1:1
		H10	Sun-dried 10 days, natural drop	12.6	....	4.6	2.5:1
		H11	Sulfured 3 hours, sun-dried 10 days	14.1	1035	4.1	2.4:1
		H12	Sulfured 3 hours, dehydrated for 24 hours at temperatures from 120° to 150°F., relative humidity from 60 to 20 per cent, air velocity 900 feet per minute	12.9	1438	3.9	2.5:1
Mission	1930	S5	Fresh, prepared as was H9	67.5	....	4.8	1:1
		S6	Sun-dried, natural drop	19.4	....	4.6	2.5:1
		S7	Picked fresh, sulfured 1 hour, sun-dried	18.3	195	4.3	2.5:1
Kadota	1932	F1	Fresh, prepared as was H9	76.2	....	4.5	1:1
		F2	Picked fresh, sulfured 7.5 hours, sun-dried	19.7	925	4.2	3.4:1
		F3	Picked fresh, dipped 90 seconds in 2 per cent boiling lye, washed, sulfured 7.5 hours, sun-dried	16.7	1157	4.2	3.5:1
Adriatic	1932	F4	Fresh, prepared as was H9	75.9	....	4.2	1:1
		F5	Picked fresh, sulfured 5 hours, sun-dried	24.7	275	4.1	3.1:1
		F6	Picked fresh, dipped 120 seconds in 2 per cent boiling lye, washed, sulfured 5 hours, sun-dried	26.3	550	4.2	3.0:1
		F7	Picked shrunken, prepared as was H9	62.9	....	4.1	1.5:1
		F8	Natural drop, sulfured 5 hours, sun-dried	14.1	256	4.3	3.5:1
		F9	Natural drop, sun-dried	15.9	....	4.3	3.5:1

A further variable lay in the dipping process, similar to that used for prunes and some raisins, to which samples F3 and F6 were subjected. This is not used in commercial production, but was tried in order to determine its effect upon vitamin retention.

Only vitamins A and C were studied in the 1929 and 1930 samples, but vitamins B and G were also included in the 1932 experiment. The biological methods used were the same as have been previously described (Morgan and Field, '30; Morgan et al., '31; Morgan et al., '35).

VITAMIN C RESULTS

None of the figs were found to be rich in antiscorbutic property, although the fresh Kadotas and Calimyrnas of 1929 were protective when daily doses of 20 to 30 gm. were given (table 2). The Kadotas of 1932 were not examined as promptly after storage as were those of 1929 and were found less active. This difference may be due to actual crop variation or to the longer frozen storage—a factor which was found (Morgan et al., '35) in grapes to exert an injurious effect. None of the other fresh fruit, Missions or Adriatics, proved protective in the amounts which could be fed. None of the dried products, sulfured or unsulfured, dipped, picked fresh or shrunken, retained measurable amounts of vitamin C.

VITAMIN A RESULTS

As is shown in table 3, the Mission figs have considerably better vitamin A endowment than have any of the lighter colored varieties. This is not surprising, since the color of the Mission fruit hints at a probably larger content of carotene. The sulfuring process was seen to increase the retention of this vitamin in the dried Mission figs, S7, sulfured, retaining about 33 per cent as compared with 20 per cent by S6, unsulfured. The same contrast may be seen in H11 and H10, dried Calimyrnas, but not in F8 and F9, dried Adriatics.

As has been demonstrated in raisins (Morgan et al., '35) and in other fruits, the dehydration process is superior to

sun-drying for preservation of vitamin A, the single dehydrated fig product, H12, Calimyrnas, being more than twice as rich as any of the sun-dried fruit, even though fresh Calimyrnas were not particularly well endowed.

The lye dipping which preceded sulfuring in F6 added no advantage as it was found to do in the case of prunes.

VITAMIN B(B_1) RESULTS

Both rat growth and pigeon maintenance methods were used in the vitamin B tests. These methods have been de-

TABLE 2

Vitamin C content of fresh and dried figs

VARIETY	LOT NO.	NUMBER OF GUINEA PIGS USED	DAILY AMOUNTS FED		AVERAGE GAIN PER DAY	LENGTH OF PERIOD	SCURVY SCORE [1]
			Grams	Equivalent in fresh			
				gm.	*gm.*	*days*	
Fresh Kadota	H9	7	10	10	—2.0	28	9
		5	20	20	0.0	43	3
		1	25	25	2.5	60	0
	F1	6	25	25	—3.1	29	14
		2	30	30	—2.3	50	12
		6	35	35	—1.8	50	12
Dried Kadotas	F2, F3	4	12	41	—4.7	33	14
Fresh Calimyrna	H8	7	7 to 15	7 to 15	—2.7	39	7
		4	20 to 30	20 to 30	3.3	60	0
Dried Calimyrnas	H10, H11, H12	37	3 to 15	7 to 37	—3.6	29	15
Fresh Mission	S5	17	5 to 20	5 to 20	—3.7	33	19
Dried Missions	S6, S7	10	4 to 10	10 to 25	—3.3	33	15
Fresh Adriatic	F4	11	25 to 30	25 to 30	—1.7	44	11
		6	35	35	—1.1	58	10
Fresh (shrunken) Adriatic	F7	16	25 to 35	37 to 42	—2.7	39	11
Dried Adriatics	F5, F6, F8, F9	8	12	36 to 42	—3.8	33	13

[1] On a scale of increasing severity of symptoms, 0 to 24.

TABLE 3

Vitamin A content of fresh and dried figs

VARIETY	LOT NO.	NUMBER OF RATS USED	DAILY AMOUNT FED		BODY WEIGHTS		PERIOD	WEIGHT YIELDING 1 INTERNATIONAL UNIT IN FRESH EQUIVALENT
			Grams	Equivalent in fresh	Initial (depleted)	Gain		
					gm.	*gm.*	*days*	*gm.*
Fresh Kadota	H9	15	0.2 to 1.5	0.2 to 1.5	66	14	28	
	F1	5	2.0	2.0	94	51	53	2.0
		5	4.0	4.0	101	77	56	
Sun-dried, sulfured Kadota	F2	5	1.0	3.4	114	34	33	
		4	2.0	6.7	121	59	56	6.7
Fresh Calimyrna	H8	13	0.15 to 0.75	0.15 to 0.75	64	6	29	
Sun-dried Calimyrna unsulfured	H10	11	0.4 to 0.9	1.0 to 2.2	72	13	33	
		4	1.0	2.5	91	14	50	
Sun-dried Calimyrna sulfured	H11	10	0.4 to 0.9	1.0 to 2.2	81	16	35	
		3	1.0	2.5	86	36	56	3.4
Dehydrated Calimyrna sulfured	H12	7	0.45	1.1	60	38	51	
		1	0.9	2.2	65	71	56	1.6
Fresh Mission	S5	6	1.0	1.0	98	46	56	1.0
		7	1.5	1.5	76	70	56	
Sun-dried unsulfured Mission	S6	9	0.9 to 1.0	2.2 to 2.5	118	5	45	
		4	1.5	3.7	115	24	56	
		6	2.0	5.0	108	42	56	5.0
Sun-dried sulfured Mission	S7	11	0.9 to 1.0	2.2 to 2.5	117	4	50	
		8	1.5	3.2	83	50	56	3.2
		5	2.0	5.0	99	61	56	
Sulfured sun-dried Adriatic	F5	3	1.0	3.1	95	0	30	
		3	2.0	6.2	93	38	50	
Dipped sulfured sun-dried Adriatic	F6	5	1.0	3.0	96	34	51	
		4	2.0	6.1	120	5	39	
Sulfured sun-dried Adriatic	F8	5	1.0	3.5	87	29	36	
		4	2.0	7.0	105	33	41	
Unsulfured sundried Adriatic	F9	4	1.0	3.5	87	21	35	
		5	2.0	7.0	86	59	56	7.0

scribed in a previous report (Morgan et al., '35). Two sources of vitamin G(B_2) were tried for the rat experiments, an autoclaved liver extract prepared under the direction of Dr. S. Lepkovsky, and the usual autoclaved yeast. The liver extract was fed separately and the yeast was incorporated in the basal diet. In general, the growth obtained with the various fig samples was much the same whichever of these vitamin G sources was used. The liver extract was used in the belief that the laxative effect of the figs and yeast together might decrease the availability of the water-soluble vitamins present. However, the liver extract was found also to produce a laxative effect upon the intestines.

The injurious effect of sulfur dioxide upon vitamin B previously recorded in the case of raisins (Morgan et al., '35) was observable in these fig tests, although in less obvious degree. As shown in table 4, containing the rat growth data, the one unsulfured dried product, F9, retained nearly twice as much of the vitamin B of the fresh fruit as did the sulfured products.

The contrast is not so marked in the pigeon maintenance results seen in table 5. It will be noted in the latter that small amounts of wheat germ were fed to supplement the fig doses in order to decrease the bulk of fruit administered. Each pigeon was standardized as to weight of wheat germ needed for maintenance of weight and prevention of disgorging or other symptoms of vitamin B deficiency. The wheat germ equivalent of the various fig preparattions could thus be rather definitely determined. In table 6 examples of the records of a few of the birds are given.

Three birds were used to determine the value of the International standard, an acid adsorbate dispensed by the Committee on Vitamin Standards of the League of Nations ('31). With the technic which has been applied in this laboratory to numerous products, based on Salmon's ('27) method, 0.05 gm. of the standard was found to be equivalent of 0.7 gm. wheat germ[1] and 0.07 gm. to be the equivalent of 1.0 gm. wheat germ.

[1] This wheat germ is a sterilized flaked product supplied by the Sperry Flour Company, of San Francisco, and found to be of constant vitamin B content when examined by both rat and pigeon tests.

TABLE 4

TABLE 4

Content of vitamin B(B_1) of fresh and dried figs

VARIETY	LOT NO.	SO_2	DAILY DOSE		NUMBER OF RATS	INITIAL (DEPLETED) WEIGHTS	TOTAL GAIN OR LOSS	PERIOD	SOURCE OF VITAMIN G	WEIGHT PROVIDING		RETENTION OF VITAMIN B OF FRESH FRUIT
			Grams	Equivalent in fresh weight						1 International unit	Sherman unit	
										Equivalent in fresh weight		
		p.p.m.		*gm.*		*gm.*	*gm.*	*days*		*gm.*	*gm.*	*per cent*
Kadota, fresh	F1	....	4.0	4.0	6	65	11	56	Autoclaved yeast	3.8	2.8	100
			6.0	6.0	3	60	53	56				
Kadota, dried	F2	925	2.0	6.7	6	69	—10	38	Autoclaved yeast			
			2.0	6.7	6	82	—22	42	Liver extract			
			3.0	10.1	5	73	—23	20	Liver extract			
	F3	1157	2.0	7.0	6	66	— 7	41	Autoclaved yeast			
			3.0	10.5	5	87	—20	28	Liver extract			
Adriatic, fresh	F4	....	2.0	2.0	3	55	4	56	Autoclaved yeast	4.0	3.0	100
			4.0	4.0	2	80	33	56	Autoclaved yeast			
Adriatic, fresh but shrunken	F7	....	2.0	3.0	7	64	14	56	Autoclaved yeast	7.3	5.3	56
Adriatic, dried	F5	275	1.0	3.1	6	66	—12	21	Autoclaved yeast	10.9	8.0	37
			3.0	9.3	6	64	29	56	Autoclaved yeast			
			1.0	3.1	6	89	—22	38	Liver extract			
			3.0	9.3	6	87	0	56	Liver extract			
Adriatic, dried	F6	550	3.0	9.2	5	86	—20	28	Liver extract			
			2.0	6.2	6	65	7	44	Autoclaved yeast			
Adriatic, dried natural drop	F8	256	1.0	3.5	6	75	—22	24	Autoclaved yeast			
			1.0	3.5	6	88	—29	43	Liver extract			
			3.0	10.6	2	68	18	56	Liver extract	16.0	12.0	25
Adriatic, dried natural drop	F9	....	1.0	3.5	6	88	—18	52	Liver extract			
			3.0	10.5	5	78	54	56	Liver extract			
			1.0	3.5	6	69	— 4	51	Autoclaved yeast	6.5	4.8	61
International standard			0.010		12	65	34	56	Autoclaved yeast	0.010	0.007	
			0.010		4	64	24	56	Liver extract	0.010	0.010	
Rice polish concentrate			0.025		14	57	43	56	Autoclaved yeast	0.020	0.014	
			0.050		15	58	102	56	Autoclaved yeast			
			0.025		5	61	34	56	Liver extract	0.025	0.019	
			0.050		4	85	99	56	Liver extract			
Nothing					23	60	— 4	25	Autoclaved yeast			
					10	85	—36	25	Liver extract			

The rice polish concentrate, prepared according to the method of Evans and Lepkovsky ('31), which is used in this laboratory as concentrated source of vitamin B practically free from vitamin G, was similarly compared on two of the same pigeons

TABLE 5

Vitamin B(B_1) content of figs as determined by pigeon maintenance test

PRODUCT	NUMBER OF BIRD	DAILY AMOUNT REQUIRED FOR MAINTENANCE					AMOUNT EQUIVALENT (FRESH) TO 0.7 GM. WHEAT GERM[1]	RETENTION OF VITAMIN B OF FRESH FRUIT
		Wheat germ alone	Weight of fruit	Equivalent in fresh weight	Additional wheat germ	Total equivalent in fresh weight		
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*per cent*
Fresh Kadota,	31	1.1	28	28	....	28	18	
F1	15	0.8	18	18	....	18	16	100
Dried Kadota,	27	1.0	8	27	0.55	60	42	
F2	33	1.0	8	27	0.55	60	42	40
Dried Kadota,	28	1.0	8	28	0.55	62	43	
F3	31	1.1	8	28	0.55	56	36	43
Fresh Adriatic,	26	1.0	15	15	....	15	10	
F4	21	0.7	15	15	....	15	15	
	29	1.2	18	18	....	18	10	
	19	0.7	15	15	....	15	15	100
Fresh shrunken	19	0.7	20	30	....	30	30	
Adriatic, F7	22	0.7	18	27	....	27	27	44
Dried Adriatic,	10	0.7	8	25	0.20	35	35	
F5	15	0.8	8	25	0.20	33	29	
	21	0.7	8	25	0.20	35	35	38
Dried Adriatic,	28	1.0	8	24	0.50	48	38	
F6	33	1.0	8	24	0.50	48	38	33
Dried Adriatic,	15	0.8	8	28	0.30	45	42	
F8	21	0.7	8	28	0.20	39	39	
	33	1.0	8	28	0.30	40	28	35
Dried Adriatic,	25	1.1	8	28	0.40	44	28	
F9	32	0.7	8	28	0.10	32	32	
	29	1.2	8	28	0.50	48	27	
	31	1.1	8	28	0.40	44	28	43
International	26	1.0	0.07				0.05	
standard	10	0.7	0.05				0.05	
	2	0.7	0.05				0.05	
Rice polish	2	0.7	0.08				0.08	
concentrate	10	0.7	0.08				0.08	

[1] Equivalent to 1 International vitamin B (pigeon) unit.

TABLE 6

Examples of pigeon maintenance test for vitamin $B(B_1)$ in figs

NUMBER OF BIRD	PRODUCT FED	DAILY AMOUNT	DATES 1932–1933	WEIGHT	CONDITION
		gm.		*gm.*	
19	Wheat germ	0.7	Oct. 1–Oct. 25	492–485	Normal
		0.6	Oct. 26–Oct.27	485–482	Normal
		0.6	Oct. 28	471	Disgorged
	Fresh fig, F4	15.0	Oct. 30–Nov. 27	478–468	Normal
		12.0	Nov. 28–Dec. 2	465	Normal
		12.0	Dec. 3		Polyneuritic
	Shrunken fig, F7	20.0	Dec. 6–Jan. 18	430–470	Normal
		18.0	Jan. 19–Jan. 24	470	
		18.0	Jan. 25	472	Disgorged
22	Wheat germ	0.7	Sept. 29–Oct. 25	430	Normal
		0.6	Oct. 26–Oct. 29	433	Normal
		0.6	Oct. 30	460[1]	Polyneuritic
	Shrunken fig, F7	15.0	Nov. 3–Nov. 16	425	Occasional disgorging
		20.0	Nov. 17–Dec. 15	420–411	Normal
		15.0	Dec. 16–Jan. 1	435–404	Disgorging
21	Wheat germ	0.7	Sept 27–Oct. 25	395–400	Normal
		0.6	Oct. 26–Nov. 10	404–476	Normal
		0.6	Nov. 11	480	Polyneuritic
	Fresh fig, F4	15.0	Nov. 19–Dec. 17	386–410	Normal
		12.0	Dec. 18–Dec. 20	412–427	Polyneuritic
15	Dried fig, F8				
		8 + 0.4 w.g.[2]	Feb. 22–Mar. 7	414–435	Normal
		8 + 0.3 w.g.	Mar. 8–Mar. 20	435–437	Normal
		8 + 0.2 w.g.	Mar. 21–Mar. 27	438	Disgorging
	Dried fig, F5	8 + 0.5 w.g.	Mar. 28–Apr. 9	430	Normal
		8 + 0.4 w.g.	Apr. 10–Apr. 22	440–415	Normal
		8 + 0.3 w.g.	Apr. 23–May 5	426	Normal
		8 + 0.2 w.g.	May 6–May 18	430	Normal
		8 + 0.1 w.g.	May 19–June 1	420	Disgorged 4 times
	Wheat germ	0.9	June 6–June 19	410–460	
		0.8	June 20–July 2	450	
		0.7	July 3–July 6	450	Disgorging
	Fresh fig, F1	30.0	Aug. 2–Aug. 14	424–426	Normal
		27.0	Aug. 15–Aug. 28	426–406	Normal
		20.0	Aug. 29–Sept. 10	406–400	Normal
		15.0	Sept. 11–Sept. 17	414	Disgorging
		18.0	Sept. 20–Oct. 2	404–410	Normal

[1] Occasionally weight increases resulted when the crop muscles were affected so that disgorging could not occur.

[2] w.g. = wheat germ.

and 0.08 gm. found to be equivalent to one International unit. Similar comparisons of the rice polish concentrate and International adsorbate units by the rat growth method yielded somewhat similar proportions, 2.0 or 2.1 to 1 by rat growth, and 1.6 to 1 by pigeon maintenance.

The amount of the various fig preparations required to protect the birds in the same degree as did the International adsorbate could thus be calculated even though the full amount required could not in all cases be fed. Apparently, the vitamin of the fresh Kadota figs was less available to the pigeons than to the rats, for the average pigeon unit amount was larger, 17 gm., than in the case of the fresh Adriatics, 12.5 gm. For the rats the unit amounts of these two kinds of figs were nearly the same, 3.8 and 4.0 gm. The loss of vitamin B in the sulfured products was entirely comparable by rat and pigeon tests in all products, except F2 and F3, the dried sulfured Kadotas. These samples appeared to be considerably better sources of the vitamin in the pigeon than in the rat tests. As was noted previously with raisins (Morgan et al., '35), greater loss of antineuritic activity with SO_2 treatment is seen when rats are used instead of pigeons as test animals. This may mean that another B vitamin, possibly B_4, is destroyed to a greater extent by the treatment than is B_1.

VITAMIN G RESULTS

The amounts of figs fed rats on a vitamin G-free basal diet proved for the most part too small to give clear cut results. The rice polish concentrate previously mentioned was given as source of vitamin B in amount of 0.05 gm. daily. All the fig products when fed at 0.5 and 1.0 gm. levels increased the growth of the animals slightly and in about the same degree, but too little to be considered significant. However, when 3 gm. doses of the dried products were fed to groups of six rats each for 56 days, a total gain of 20 to 40 gm. was noted. No significant differences among the samples could be said to emerge. The fresh fruit was not fed at this higher level. The tentative conclusion may be drawn that the dried figs

contained from 1 to 1½ Sherman units of vitamin G in 3 gm.—a value not affected measurably by sulfuring or other variations in the drying procedure.

The chief choices involved in the drying of fruits appear to be sun-drying versus dehydration and sulfuring versus no sulfuring. If one regards only the effect of these processes upon the vitamin the choice is somewhat complicated by the fact that some vitamins are affected advantageously by one choice and others by the opposite choice. Thus vitamins A and C are best preserved by removal of oxidative hazards, that is by the use of dehydration instead of sun-drying and by the employment of sulfur dioxide. Vitamin B appears to be better protected by quite the opposite conditions, sun-drying and no sulfuring. However, dehydration instead of sun-drying is far less injurious to vitamin B than is sulfuring. Vitamin G is apparently unaffected by any of these processes. Fruits rich in vitamins A and C should, therefore, preferably be dehydrated and possibly also sulfured, those rich in B sun-dried and not sulfured. Mission figs should be dehydrated to preserve their vitamin A value, but Kadotas, Calimyrnas and Adriatics, poor in A, should be sun-dried and not sulfured in order to retain their good vitamin B value.

SUMMARY

1. Kadota and Calimyrna fresh figs, preserved in frozen storage, were found to contain vitamin C in measurable amounts, similar to that found in grapes and apricots, but less than that in fresh peaches and fresh prunes. Mission and Adriatic figs contained but little of this vitamin.

None of the dried figs, sulfured or unsulfured, sun-dried or dehydrated, retained measurable antiscorbutic value.

2. The Black mission figs contain considerably more vitamin A precursor than do any of the light colored figs, 100 International units per 100 gm. fresh as compared with 50 in fresh Kadotas and less in the Calimyrnas. The sulfuring process proved favorable to the retention of vitamin A of the Missions and Calimyrnas, but not of the Adriatics. The one de-

hydrated product, Calimyrnas, H12, was far superior to all the sun-dried samples, as was previously found true of dehydrated raisins and other dried fruits. The dried figs contained 50 to 143 International units of vitamin A per 100 gm.

3. Vitamin B(B_1) tested by the rat growth method appeared to be unfavorably affected by sulfuring preceding sun-drying. Fresh Kadotas and Adriatics contained about equal amounts of the vitamin, 25 International or 35 Sherman units per 100 gm. The unsulfured dried Adriatics retained 61 per cent of this and all sulfured products 37 per cent or less, that is 71 and 33 Sherman units per 100 gm. of dried fruit.

By pigeon maintenance test much the same relations were discovered, except that the destruction of the vitamin in the sulfured fruit was less obvious. This may be due to more complete destruction of a third B vitamin needed by rats and not by pigeons.

4. Vitamin G is present in figs, the dried products containing practically the same amount in all cases, 33 to 50 Sherman units per 100 gm. None of the variables of the drying process appeared to affect this vitamin.

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THE VITAMIN B AND G CONTENT OF PRUNES

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ONE CHART AND ONE FIGURE

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The problem of the effect of various methods of drying upon the vitamin content of fruit has been studied during several years in this laboratory. Certain conclusions have been drawn as to the protective value of dehydration instead of sun-drying, and of sulfuring upon vitamins C and A in peaches, prunes and apricots (Morgan and Field, '29, '30; Morgan, Field and Nichols, '31). No attempt was made until recently to assess the vitamin B(B_1) and G(B_2) value of these dried fruits. The present report presents the result of such determinations upon samples of California prunes.

Eddy, Gurin and Kohman ('32) tested canned prunes, both the French and Italian varieties for vitamins B and G. Their results showed very little content of B in their samples—a fact which cannot be ascribed properly to lack of this factor in the fruit, since the canning process is known to decrease antineuritic value. They made no tests upon corresponding fresh fruit. The vitamin G activity shown by their canned specimens was comparable with that here reported for the dried fruit. The only other report upon the B content of prunes is that made by Osborne and Mendel ('20) upon the B complex. Their limited data apparently indicate nearly normal growth of rats on 2 to 5 gm. of prune per day—a better performance than was obtained on any of the other fruits which they examined.

The prunes used in the work here reported were purchased in the open market, there being no specification mentioned, except that they be of California origin and of a medium size, fifty to sixty per pound. Several different 25-pound boxes were purchased and no attempt was made to segregate the results obtained with these different lots. They were kept in a cool dry place in the laboratory. The fruit contained 22 to 25 per cent water.

Methods. In general, the technic described by Chase and Sherman ('31) was followed for the vitamin B determinations on rats and that of Bourquin and Sherman ('31) for the vitamin G assay.

The vitamin G tests. The source of vitamin B used in the vitamin G tests was not that recommended by Chase and Sherman, but was a potent rice polish extract made according to the technic of Evans and Lepkovsky ('31). This preparation was found to be rich in the antineuritic property and when made from the polish only, without the rice bran, was almost devoid of vitamin G. Daily doses of 0.05 cc. were found sufficient for practically normal growth when vitamin G was supplied.

Both 28- and 56-day feeding periods were used with results indicating that either period is acceptable, but that, as might be expected, the growth increments per week found in the shorter time are greater than the corresponding values for the longer period. The ratios of the increments produced by the various doses, however, are nearly the same in the 28- and 56-day tests. Some general agreement as to the length of the test period to be used for vitamin G assays is urgently needed in order to make the data from different laboratories comparable.

As may be noted in table 1 and chart 1, the growth increments produced by increasing daily allowances of prunes are not arithmetically proportional to these allowances. Moreover, the rats do not die within the longer experimental period of 56 days when confined to the vitamin G-free diet nor does their weight decline below the initial figure. The

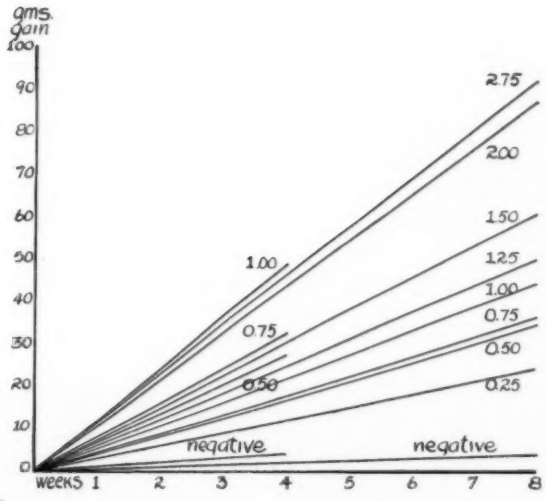

Chart 1 Gains in weight of young rats fed varying amounts of prune as source of vitamin G. The daily doses are given in grams opposite the growth angles.

TABLE 1

Vitamin G content of prunes

DAILY AMOUNT FED	NUMBER OF RATS	BODY WEIGHTS			NUMBER OF RATS	BODY WEIGHTS		
		Initial	Final	Gain in 28 days		Initial (depleted)	Final	Gain in 56 days
gm.		*gm.*	*gm.*	*gm.*		*gm.*	*gm.*	*gm.*
0.25					19	66	91	25
0.50	16	55	83	28	21	65	100	35
0.75	14	51	84	33	12	59	96	37
1.00	8	63	112	49	13	58	103	45
1.25					15	56	107	51
1.50					14	54	116	62
2.00					16	52	140	88
2.75					4	58	151	93
Negatives	4	59	65	6	6	64	68	4
	3	49	53	4	14	70	76	6
					21	58	63	5

average gain of forty-one such rats in 8 weeks was 5 gm. Severe dermatitis with loss of fur and sometimes regrowth of soft thin hair, nose bleed, tail scale and blindness were seen in nearly all such animals. Several animals were kept for 6 to 15 months on the vitamin G-free diet. Severe convulsions on disturbance were common among these rats after several months on the diet. One pair of littermates was kept for 18 months, one being given only the G-free diet and the other in addition 2 gm. of prune flesh daily. Figure 1 illustrates their appearance after 7 months on the diet.

Since no generally accepted standard is as yet available for vitamin G assays, the results shown in table 1 must be

Fig. 1 Prunes as source of vitamin G. Rats of same litter on vitamin G-free diet for 7 months, that on the right being given 2 gm. prune flesh daily in addition to basal.

summarized in the units suggested by Bourquin and Sherman ('31), that is, the daily amount which produces an average gain of 25 gm. in 8 weeks. This may be taken as 0.25 gm. of prunes at the least and 0.50 gm. at the most, about 266 units per 100 gm. or 75 units per ounce.

Using the same technic in this laboratory, certain dried foods, reputed to be highly endowed with vitamin G, have been assayed for comparison with the prune results. The data are shown in table 2. It will be noted that prunes are nearly equal to wheat germ and are reasonably close in value to the solids of milk and eggs. The solids of spinach contain two to three times this amount of the vitamin and those of brewery yeast and beef liver about four times as much. No

other fruit tested in this laboratory approaches prunes in this respect.

The vitamin B results. Most of the vitamin B tests were made with a basal diet consisting of purified casein 18 per cent, Osborne and Mendel salt mixture 4 per cent, starch 53 per cent, butterfat 8 per cent, cod liver oil 2 per cent, and yeast (autoclaved for 6 hours at 15 pounds' pressure) 15 per cent. In certain cases, however, the yeast was omitted

TABLE 2

Vitamin G content of certain dried concentrates

FOOD	DAILY AMOUNT FED	NUMBER OF RATS	INITIAL (DEPLETED) WEIGHT	FINAL WEIGHT	GAIN IN 56 DAYS	SHERMAN UNITS PER GRAM
	gm.		*gm.*	*gm.*		
Prunes	0.25	19	66	91	25	4.0
	0.50	21	65	100	35	2.8
Wheat germ	0.25	4	53	85	32	4.1
	0.50	15	65	112	47	3.7
Dried whole eggs	0.50	8	68	124	56	4.5
Dried whole milk	0.25	8	61	96	35	5.6
	0.50	7	77	128	51	4.1
Dried spinach	0.25	8	62	124	62	9.9
	0.50	4	67	170	103	8.2
Dried brewery yeast	0.12	2	74	135	61	19.5
	0.25	8	67	156	89	14.2
Dried beef liver	0.12	4	69	116	47	15.0
	0.25	18	59	158	99	15.8

with corresponding increase in starch and a potent liver extract used instead as source of vitamin G. This was done in the hope of discovering whether the laxative effect of the prunes augmented by the similar yeast effect may have resulted in a disproportionate intestinal loss of their vitamin value. The liver extract, which was made and autoclaved under the supervision of Dr. S. Lepkovsky, was shown to be rich in vitamin G and poor in vitamin B. Nevertheless, poorer growth was seen in the rats fed this diet than in com-

parable animals given the diet containing autoclaved yeast. As may be seen in tables 3 and 4, the International standard preparation as well as our rice polish concentrate yielded poorer growth when the liver extract was used. Apparently, the vitamin G content of the liver extract allowance (0.4 cc. daily) was insufficient, or some other condition, possibly an effect on appetite, or lack of a third vitamin B factor, interfered with the welfare of these animals.

TABLE 3

Vitamin B content of prunes (with autoclaved yeast as source of vitamin G)

SUBSTANCE	DAILY AMOUNT FED	NUMBER OF RATS	INITIAL (DEPLETED) WEIGHT	FINAL WEIGHT	GAIN	PERIOD
	gm.		*gm.*	*gm.*	*gm.*	*days*
Prunes	0.75	8	49	71	22	56
	1.00	17	63	88	25	56
	1.25	21	62	90	28	56
	1.50	23	56	90	34	56
	2.00	8	58	103	45	56
	2.75	10	62	113	51	56
	0.00	23	50	46	—4	25
Rice polish concentrate	0.025	14	57	100	43	56
	0.050	15	58	160	102	56
	0.075	4	76	213	137	56
	0.100	4	74	221	147	56
International standard	0.005	4	75	67	—8	56
	0.010	18	68	102	34	56
Wheat germ	0.15	9	60	103	43	56
Whole wheat	0.6	9	62	93	31	56
	0.8	6	78	137	59	56

Three different lots of vitamin B acid clay adsorbate used as International standard, and received from Dr. E. M. Nelson, of the United States Department of Agriculture, were tested for growth stimulation. In no case did the unit amount, 10 mg., produce the nearly normal growth expected from the published reports of similar tests by Chick and Jackson ('32) and Kemmerer and Steenbock ('33). However, in both the latter experiments the feeding period was only 4 or 5 instead

of 8 weeks. A similar low antineuritic value was noted when the adsorbate was fed to pigeons using the prophylactic method of Salmon ('27).

If our results with the International standard be compared with those obtained with the rice polish concentrate, the latter may be seen (table 3) to contain 1 unit of vitamin B in 0.02 gm. The prunes which we used contained 1 such unit in 1.5 gm. If these values be expressed in Sherman units (the amount

TABLE 4

Vitamin B content of prunes (with liver extract as source of vitamin G)

SUBSTANCE	DAILY AMOUNT FED	NUMBER OF RATS	INITIAL (DEPLETED) WEIGHT	FINAL WEIGHT	GAIN	PERIOD
	gm.		*gm.*	*gm.*	*gm.*	*days*
Prunes	1.00	18	60	51	—9	39
	1.50	10	64	84	20	54
	2.00	21	69	77	8	56
	2.75	8	74	102	28	56
Nothing		9	75	50	—25	18
Rice polish concentrate	0.025	5	61	95	34	56
	0.050	14	66	139	73	56
International standard	0.005	7	73	70	—3	56
	0.010	4	64	88	24	56

allowing 25 gm. gain in rats during 8 weeks), the figures are 0.015 and 1.0 or 1.25 gm. This indicates 22 to 28 Sherman units per ounce or 80 to 100 per 100 gm. The results obtained with wheat germ and whole wheat in a parallel series of tests are quoted in table 3 for comparison and evaluation of the method.

SUMMARY

1. The vitamin G(B_2) content of dried California (French) prune flesh is at least 266 Sherman units per 100 gm.. This is larger than has been reported for any other fruit, is comparable with the value found for wheat germ and is one-half to one-fourth that of dried milk, eggs, spinach, yeast and liver.

2. Both 28- and 56-day periods are suitable for vitamin G feeding experiments, but the growth increments obtained with one period are not comparable with those of the other.

3. The vitamin B(B_1) content of the dried prune flesh is 80 to 100 Sherman units per 100 gm. Since the International standard adsorbate in our hands yielded only 34 gm. gain in 8 weeks per 10 mg. (1 unit), the prunes may be said to contain 1 International vitamin B unit in 1.5 gm. This is approximately one-tenth the amount found in wheat germ and nearly one-half that of whole wheat.

4. A liver extract substituted as source of vitamin G(B_2) for the autoclaved yeast in some of the B_1 tests allowed less growth than did the yeast-containing diet, although its vitamin G content appeared adequate. Possibly a third B factor is involved in this difference.

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